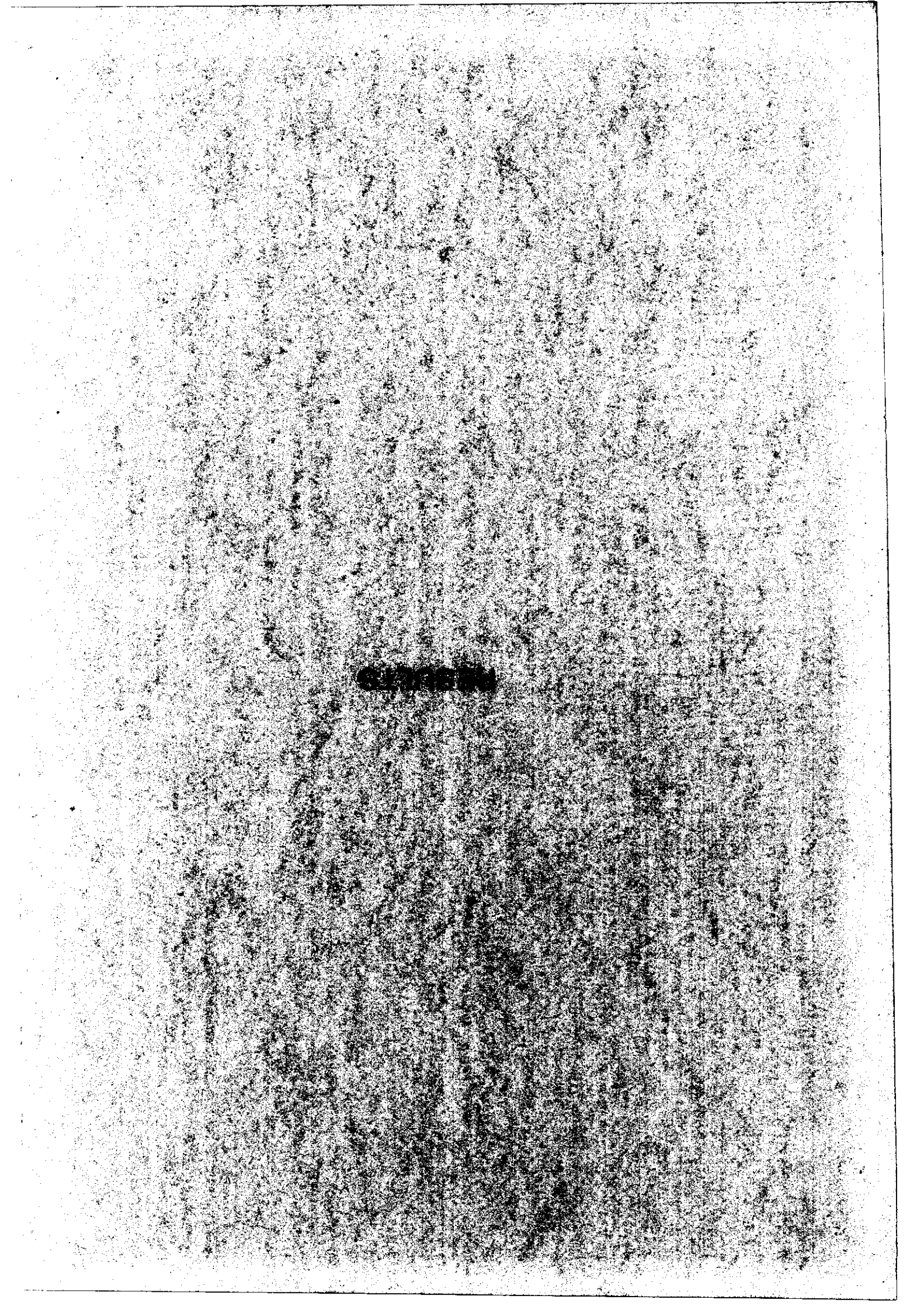


RESULTS

Histology, histochemistry and cytophotometric analysis :

I- Ovary :

1- Histological changes :

Treatment with the insecticide resulted in different changes both in the follicles and stroma compared to control (Fig. 2). Even in 0.5 mg treated fishes for 5 days, the ovarian stroma shows some degenerative changes (Fig. 3). Follicular collapse can be observed in 1.0 mg/L treated fish for 5 days (Fig. 4) and is quite evident in 0.5 mg/L treated fishes for 10 days (Fig. 5). The large ovarian follicles show nuclear and cytoplasmic irregularities in ovaries of treated fish with 1.0 mg/L for 10 days (Fig. 6). Such changes are highly evident after 15 days of treatment with 0.5 mg/L (Fig. 7) and 1.0 mg/L sevin in which nuclear degeneration is clear in some follicules (Fig. 8).

Transverse section in ovary of <u>Oreochromis niloticus</u> :

Fig. (2): control, N = Nucleus, n = nucleoli, 0 = ooplasm.

Fig. (3): 0.5 mg/L treateflor 5 days, Sd = stromal degeneration.

Fig. (4): 1.0 mg/L treated for 5 days, FC: follicular collapse.

Fig. (5): 0.5 mg/L treated for 10 days, FC: follicular collapse.

Fig. (6): 1.0 mg/L treated for 10 days.

Fig. (7): 0.5 mg/L treated for 15 days.

Fig. (8): 1.0 mg/L treated for 15 days.

(Hx & E. X 200).

Stages of ovarian cycle :

The different stages of ovarian maturation in **Oreochromis niloticus** were followed morphologically and according to their stainability with Milligan trichrome stain. A colour index was developed using this stain in which the different stages of follicular maturation appeared in diffferent colours as follows (Fig. 9,10) :

First stage : red

Second stage : magenta

Third stage : violet-magenta

Fourth stage : Violet

Fith stage (mature follicle) green.

Atretic follicles:dense green with red peripheral rim

The ratio of each stage relative to other follicular forms was quantitatively followed :

In 0.5 man sevin treated fish, this ratio was found as follows (table 1,1 Fig. 23.11.12.15,16,19,20) :

1- The primary egg cell stage :

It's ratio was less in treated compared to control at 5,10 and 15 days.

The lowest value was at 10 days followed by 5 days then 15 days.

2- Second stage follicle :

It's less in both 5 and 10 days but was higher at 15 days than the control.

3- Third stage :

It is simillar to the 2nd stage.

4- Fourth stage :

In contrast to the earlier stages, treatment with the insecticide results in higher percentage of this stage relative to control.

The highest percentage was at 5 days followed by 10 days then 15 days.

5- Fifth stage :

The relative percentage of this stage is less in treated fish than control. Although the values were almost equal after 5 and 10 days of treatment, a much smaller percentage was found after 15 days of treatment.

6- Atretic follicles :

The percentage of atretic follicles is very high after 5 and 10 days of treatment. However, after 15 days, the percentage is even smaller than control.

In 1 mg/L sevin treated fish the ratio of the different stages (table 1,Fig. 24,13,14,17,18,21,22) was as follows:

1- The primary egg cell stage :

The percentage was less in all treatments than that of control. The least value was obtained after 10 days of treatment. The percentage of the primary cell stage was, however, less after 15 days than 5 days.

2- Second stage follicle :

Although the percentage of these follicles after 5 days of treatment is higher than control,it is significantly lower after 10 and 15 days of treatment.

3- Third stage :

Generally, the percentage of these follicles is a less in treated than control, the lowest valaue was obtained after 10 days of treatment.

4- Fourth stage :

There is a duration dependent increase in the percentage of these follicles in all treated than that of control

5- Fifth stage :

Generally the percentage of follicles in this stage is less in treated than control,the lowest value was obtained after 15 days of treatment.

6- Atretic follicles :

There is a higher percentage of atretic follicles in the ovaries of fish incubated in the insecticide for 5 and 10 days. The percentage, however, is equal to control after 15 days of treatment.

Transverse sections in ovary of **Oreochromis niloticus** (Malligan trichrom).

Fig. (9): Control, 1-first stage of follicle, 2-second stage, 3-third stage, 4-fourth stage, 5-fifth stage, A-atretic follicle (Malligan trichrom X 40).

Fig. (10): Control (X100).

Fig. (11): 0.5 mg/L treated for 5 days (X40).

Fig. (12): 0.5 mg/L treated for 5 days (X100).

Fig. (13): 1.0 mg/L treated for 5,days (X40).

Fig. (14): 1.0 mg/L treated for 5 days (X100).

Fig. (15): 0.5 mg/L treated for 10 days (X40).

Fig. (16): 0.5 mg/L treated for 10 days (X100).

Fig. (17): 1.0 mg/L treated for 10 days (X40).

Fig. (18): 1.0 mg/L treated for 10 days (X100).

Fig. (19): 0.5 mg/L treated for 15 days (X40).

Fig. (20): 0.5 mg/L treated for 15 days (X100).

Fig. (21): 1.0 mg/L treated for 15 days (X40).

Fig. (22): 1.0 mg/L treated for 15 days (X100).

Table (1): The average of the stages of the maturation cycle of the ovary of 0. niloticus

Days	Control	Treated animal with 0.5 mg/L sevin	Treated animal with 1.0 mg/L sevin	Stages
5 days	30.28	11.81	22.44	(1)
	23.12	15.81	25.13	(2)
	21.13	11.64	14.64	(3)
	11.60	37.29	21.60	4
	6.5	4.5	5.92	(5)
	7.1	18.8	14.1	Atretic stage
10 days	30.28	9.91	8.73	(1)
	23.71	17.41	18.61	(2)
	21.13	13.07	10.25	(3)
	11.30	29.49	28.74	(4)
	6.50	4.70	4.5	(5)
	6.75	24.89	17.31	Atretic stage
15 days	30.28	20.57	16.94	(1)
	23.71	26.11	17.03	(2)
	21.13	29.59	14.90	(3)
	6.5	18.62	31.79	(4)
	7.06	0.99	1 . 1	(5)
		2.56	6.90	Atretic stage

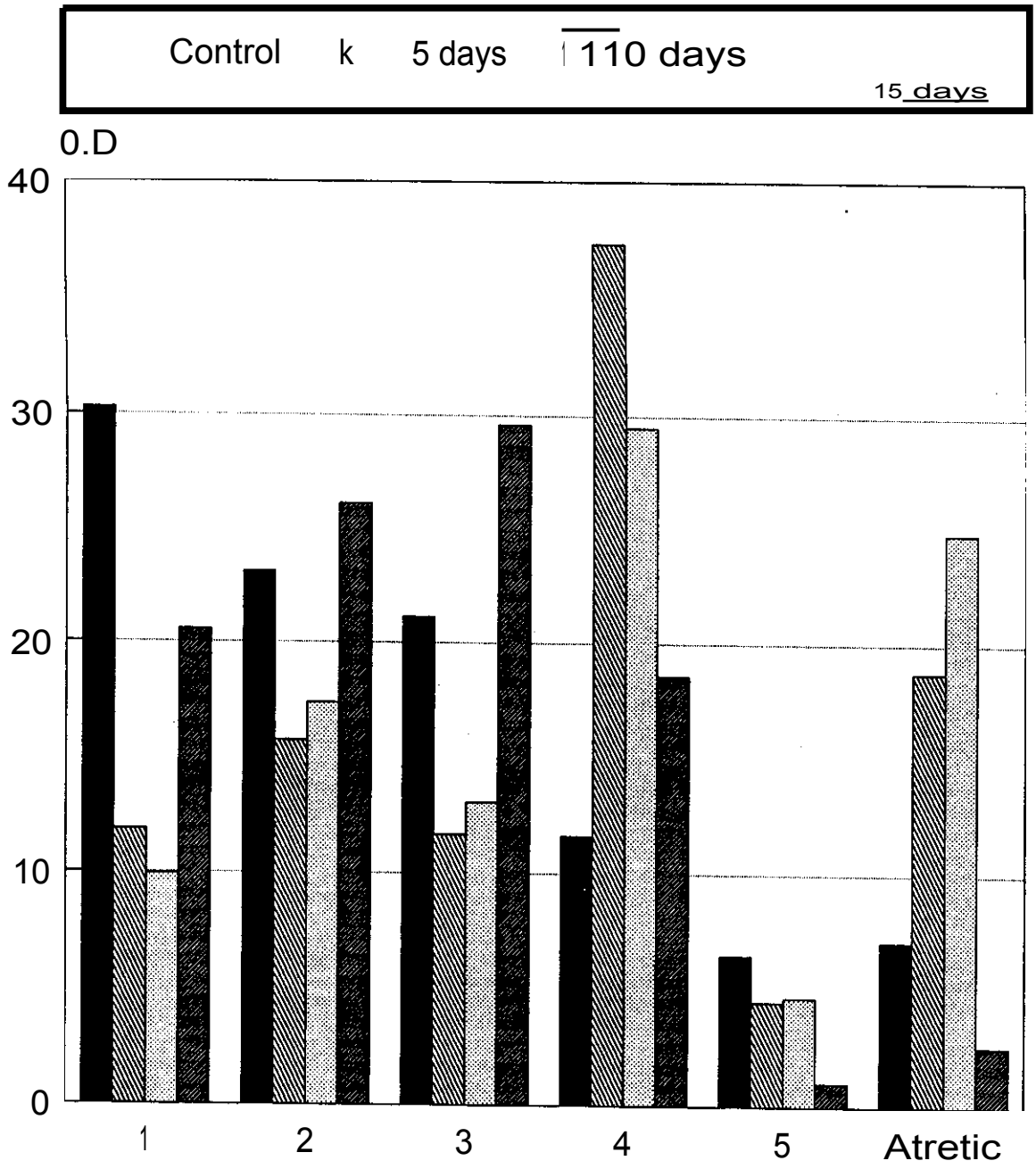


Fig.(23,): Average stages of the ovarian cycle of **Oreochromis niloticustreated with** 0.5 mg/L Sevin

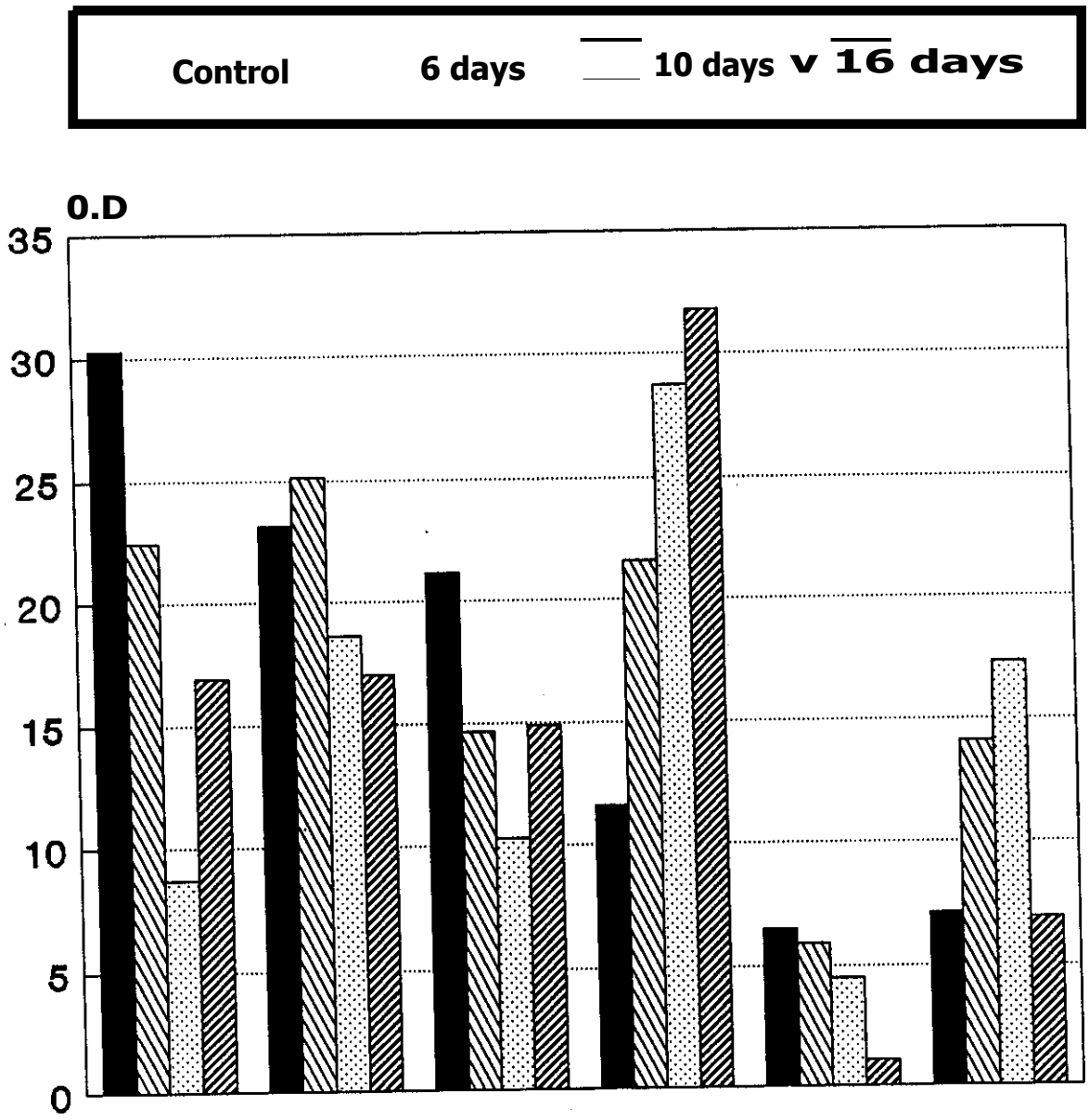


Fig.(24): Average stages of the ovarian cycle of Oreochromis niloticus treated with 1.0 mg/L Sevin.

Cytophotometric measurement of glycogen in ovary (table 2, Fig. 25) :

The amount of glycogen per cell as demonstrated by PAS stain is generally different in treated animal Oocytes than that of control (Fig. 26-31).

The value obtained for fishes treated with 0.5 mg/L sevin for 5 days (0.55 ± 0.16) is much smaller than that of the control (1.12 ± 0.19). After 10 days of treatment with this dose, the value obtained (1.09 ± 0.194) was non significantly less than that of the control and after 15 days of treatment the optical density (1.24 ± 0.24) was also non significantly higher than control.

Treatment with 1 mg/L gave values of (1.69 ± 0.22) at 5 days and (2.16+0.22) at 10 days which are significantly higher than of the control. The value, however, was (0.88+ 0.30) after 15 days which is significantly high as compar-ed to the control group for which the value was (1.12 ± 0.19).

Table (2): Effect of sevin on PAS positive materials in the ovary, of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean + s.D	1.12 0.19	0.55* 0.16	1.69* 0.22
10	Mean + S.D	1.12 0.19	1.09 0.194	2.16* 0.22
15	Mean + S.D	1.12 0.19	1.24 0.24	0.88* 0.30

* Significant difference as compared to control at P< 0.025 and Ps 0.05 (t) test.

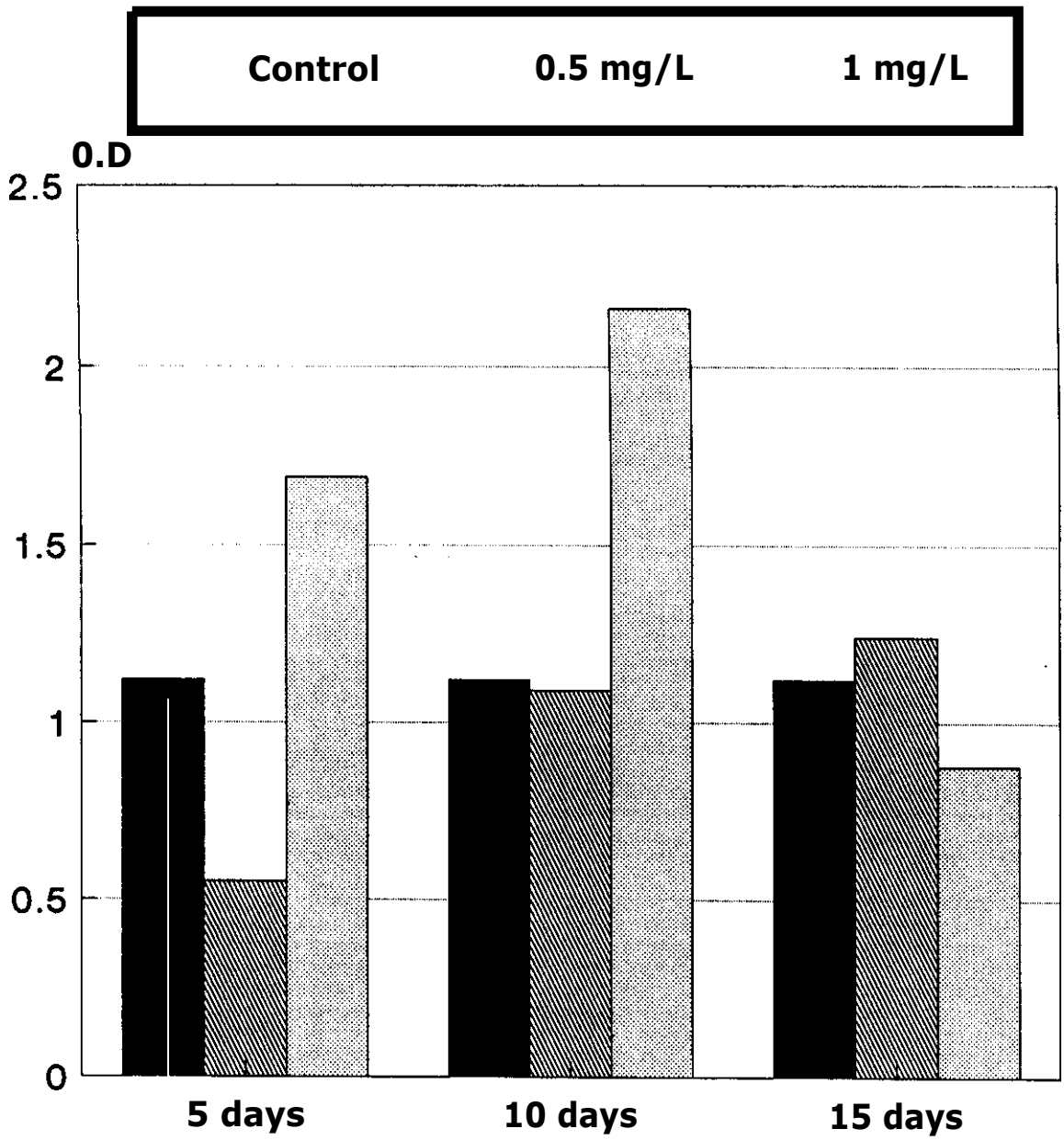


Fig. (25): The mean optical density values of glycogen content (PAS) in ovary of Oreochromis niloticus•

Transverse sections in ovary of **Oreocbromis niloticus** (PAS-Methyl gree).

Fig. (26): Control (X200)

Fig. (27): Control. (X400)

Fig. (28): 0.5 mg/L treated for 5 days. (X200)

Fig. (29): 0.5 mg/L treated for 5 days. (X400)

Fig. (30): 1.0 mg/L treated for 5 days. (X200)

Fig. (31): 1.0 mg/L treated for 5 days. (X400)

Fig. (32): 0.5 mg/L treated for 10 days. (X200)

Fig. (33): 0.5 mg/L treated for 10 days (X400)

Fig. (34): 1.0 mg/L treated for 10 days. (X200)

Fig. (35): 1.0 mg/L treated for 10 days. (X400)

Fig. (36): 0.5 mg/L treated for 15 days. (X200)

Fig. (37): 0.5 mg/L treated for 15 days. (X400)

Fig. (38): 1.0 mg/L treated for 15 days. (X200)

Fig. (39): 1.0 mg/L treated for 15 days. (X400)

Cytophotometric measurement of RNA in ovary (table 3 Fig. 40):

The amount of RNA per cell as demonstrated by pyronin stain is generally higher in treated animal oocytes than that of the control (Fig. 41 - 61).

Treatment with 0.5 mg/L sevin resulted, in the highest optical density value relative to RNA content after 5 days (0.595 + 0.165), 10 days (0.61 + OA%) and 15 days (0.56 0.18).

Treatment with 1 mg/L comes next to the first treatment with value of (0.34 +0.205) at 5 days, (0.4 +0.16) at 10 days and (0.495 ± 0.15) at 15 days as compared to the control group for which the value was 0.32 ± 0.25.

Table (3): Effect of sevin on RNA content in ovary of O. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.32 0.25	0.595* 0.165	0.34 0.205
10	Mean + S.D	0.32 0.25	0.61* 0.18	0.44* 0.16
15	Mean ± S.D	0.32 0.25	0.56* 0.18	0.495* 0.15

* Significant difference as compared to control at $P< 0.025$ and $P< 0.05$ (t) test.

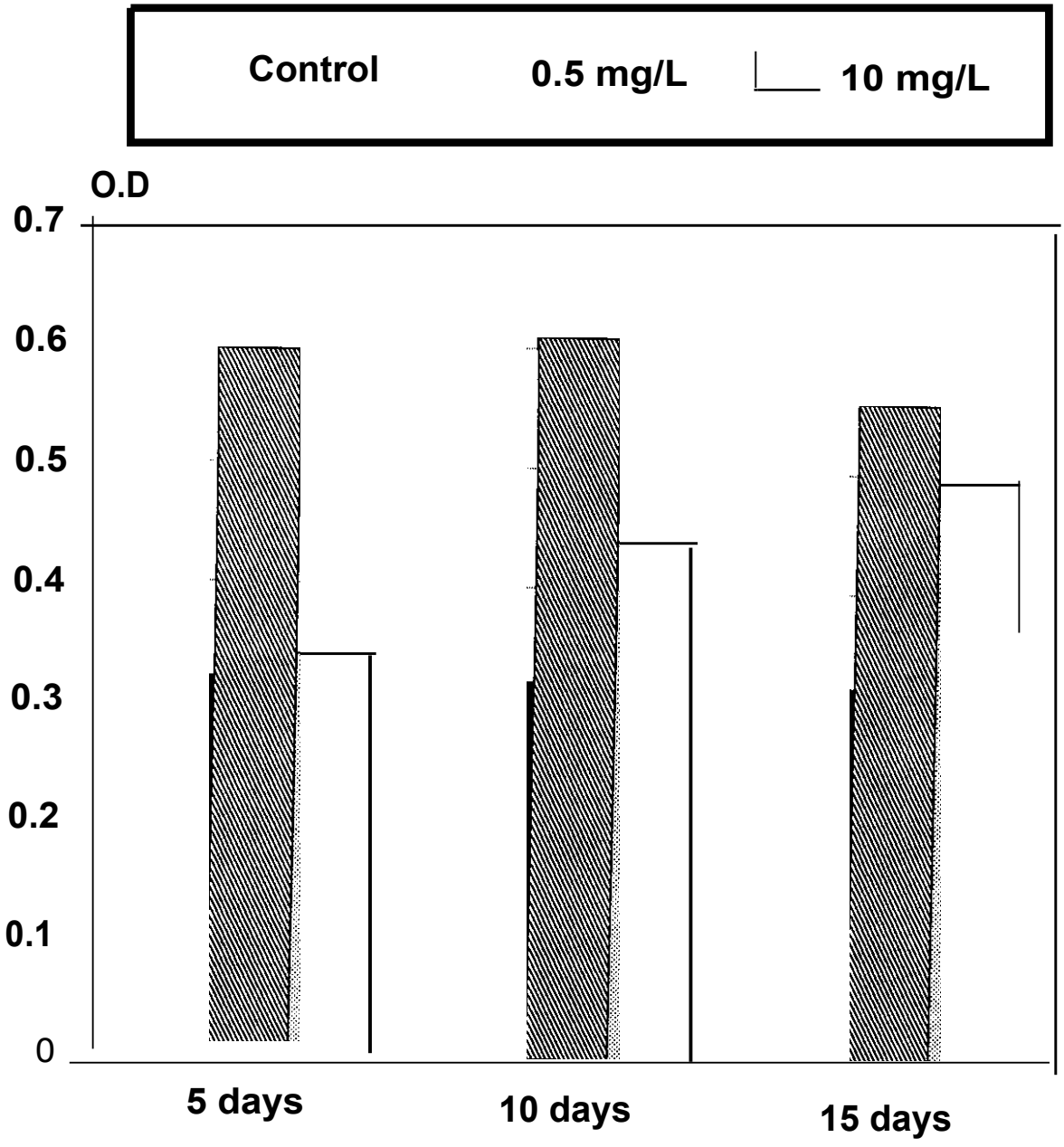


Fig.(40):The mean optical density (O.D.) values of RNA content in ovary of Oreoch rom is niloticus•

Cytophotometric measurement of DNA in ovary(Feulgen stain) (table 4, Fig. 62,63):

Nuclear DNA content per ovarian cell of 0.5 mq/L treated fish is slightly but significantly higher than that of the control after 5 and 10 days of treatment. However, after 15 days the amount in treated fish cells is almost equal to that of the control.

on the other hand, the amount of DNA per cell it the ovary Of 1.0 ing/L treated fish i% that that of e control after 5,10 and higher meet. However 15 days of treatdays of treatment. the value is comparatively low after, 15

The frequency distribution analysis of Feulgen stained ovarian cells indicates difference in the effect of different doses on DNA content. In the O. 5 mg/L at 5 days there are 3 population of cells: One with DNA content lower than that of the control, another with DNA content equal to that of the control and a third population of cells containing higher DNA content. The 1.0 mg/L treated, however, has a population of cells with DNA content equal to the high values of 0.5 mg/L and another population wit with even higher DNA content.

After 10 days the 0.5 mg/L treated fish cells has most its population equal to that of the control,but still another population of cells higher than control. The 1.0

mg/L, however, shows a population of cells that have a much higher build up of DNA per cell.

After 15 days of treatment, although most of the cells of 0.5 mg/L treated fish show population just higher than the normal, the amount of DNA per nucleus as demonstrated by Feulgen stain is generally higher in treated animal Oocyte than that of the control,

Table (4): Effect of Sevin on DNA content in ovary (Fulgen), of 0. nilotiCuS.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.35 0.068	0.38* 0.27	1.07* 0.31
10	Mean ± S.D	0.35 0.068	0.47* 0.16	2.01* 0.29
15	Mean ± S.D	0.35 0.068	0.36 0.205	0.79* 0.13

* Significant difference as compared to control at Pc_0 .025 and Pc 0.05 (t) test.

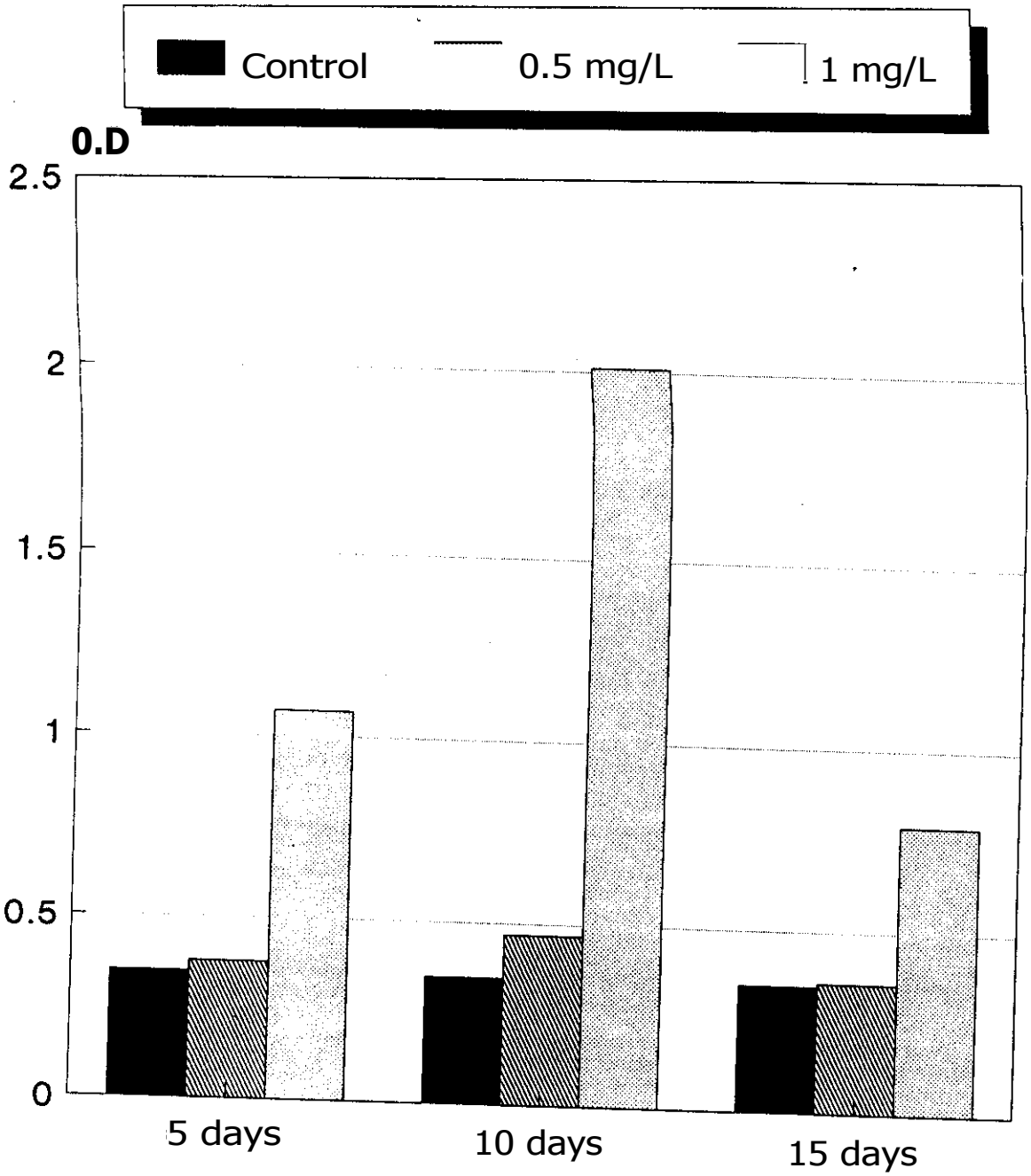


Fig.(62):The mean optical density value of DNA content (Feulgen stain) in ovary of **Oreochromis niloticus**•

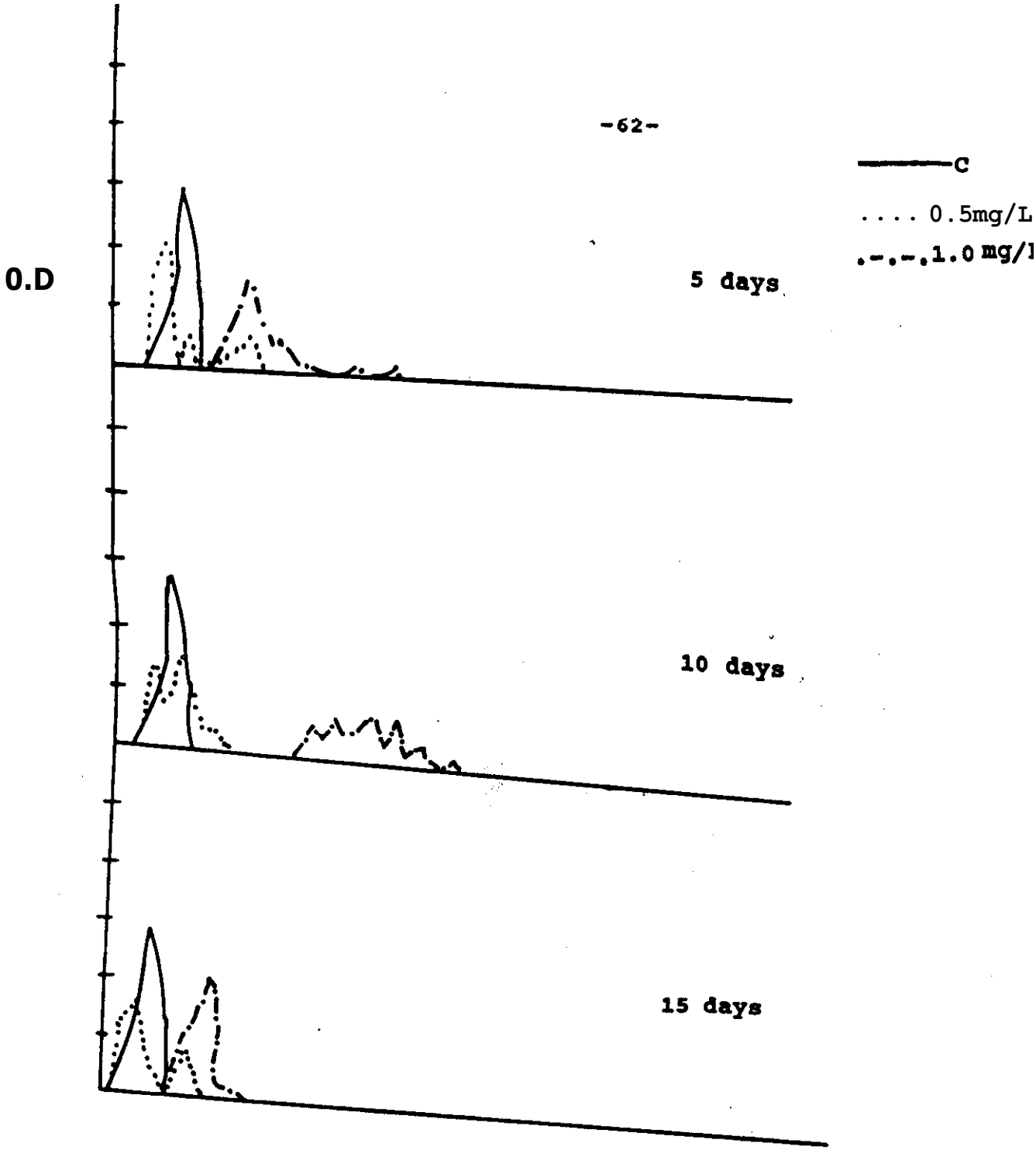


Fig. (63): Frequency distribution histogram of the mean optical density of DNA (feulgen stain) in ovarian cells or UStreated with o.5 and 1.0 mg/1 sevin for 5,10 and 15 days.

Cytophotometric measurement of keratin in ovary (Table 5, Fig. 64) :

The amount of keratin per cell as demonstrated by performic acid Schiff's stain appear different in treated animal Oocytes as compared to that of the control. In both 0.5 mg/L and 1.0 mg/L treated fish, the amount of keratin per ovarian cell is significant from that of the control only after 15 days of treatment.

In 0.5 mg/L treated fish, the mut* of keratin is almost equal to that of control (0.574 ± 0.19) after 5 days, (0.57 ± 0.24) nonsignificantly higher after 10 days (0.59 ± 0.17) and significantly lower after 15 days of treatment (0.405 ± 0.13).

After treatment with 1.0 mg/L Sevin the amount of keratin per cell is less after both 5 days (0.51 ± 0.16) and 10 days (0.48 ± 0.28) but significantly high after 15 days of treatment (0.71+0.26) as compared to the control.

Table (5): Effect of Sevin on keratin content in ovary, of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.574 0.19	0.57 0.24	0.51 0.16
10	Mean ± S.D	0.574 0.19	0.59 0.17	0.48 0.28
15	Mean ± S.D	0.574 0.19	0.405* 0.13	0.71* 0.26

* Significant difference as compared to control at P< 0.025 and P.< 0.05 (t) test.

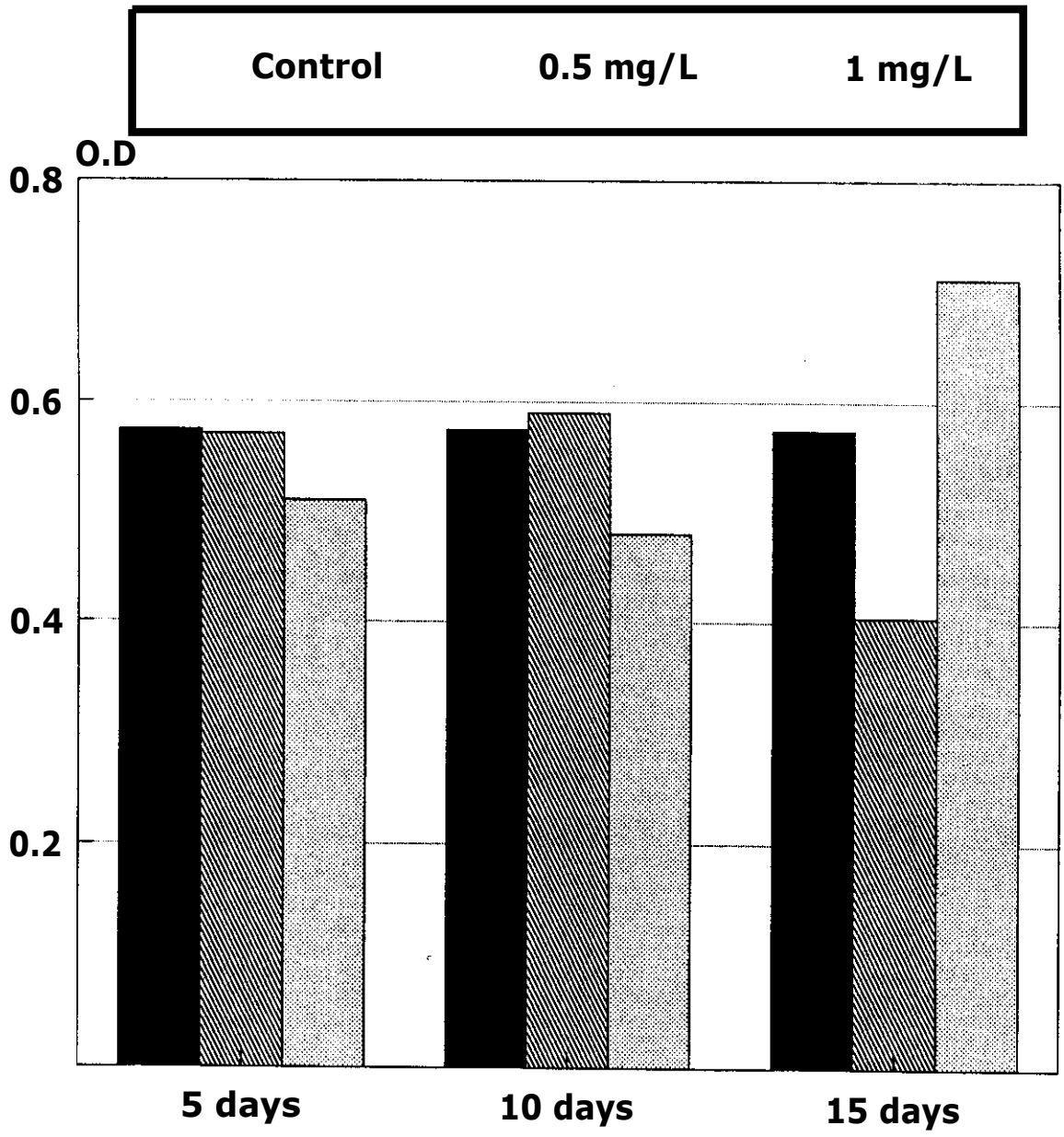


Fig. (64): The mean optical density (O.D.) values of keratin content in ovary of <u>Oreochromis niloticus</u>●

Cytophotometric measurement of Amino group rich proteins in ovary (Table 6, Fig. 65) :

The amount of amino group rich protein per ovarian cell is nonsignificantly different from control value in 0.5 mg/L treated fish.

In 1.0 mg/L treated fish, the amount of amino group rich protein per ovarian cell is significantly less than control after 5 days of treatment. However, the values obtained after 10 and 15 days of treatment are statistically nonsignificant from control.

Table (6): Effect of Sevin on Amino group rich proteins content in vary of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	1.66 0.36	1.65 0.52	1.01* 0.14
10	Mean ± S.D	1.66 0.36	1.82 0.384	1.68 0.38
15	Mean ± S.D	1.66 0.36	1.61 0.45	1.65 0.60

* Significant difference as compared to control at $P< 0.025$ and $P< 0.05$ (t) test.

Cytophotometric measurement of SS. group rich proteins in ovary (Table 7, Fig. 66) :

The amount of SS. group containing proteins per cell as demonstrated by performic acid alcian blue stain is significantly less in 0.5 mg/L treated animal oocytes at 5 days and almost equal to that of the control at 10 days; but significantly high at 15 days as compared to that of the control. It is significantly high in 1.0 mg/L treated group at both 5 and 10 days and almost equal to the control at treatment.

Table (7): Effect of Sevin on SS. group rich proteins content in ovary of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.43 0.05	0.34* 0.12	0.56* 0.04
10	Mean ± S.D	0.43 0.05	0.40 0.12	0.53* 0.03
15	Mean ± S.D	0.43 0.05	0.63* 0.03	0.42 0.06

* Significant difference as compared to control at Ps 0.025 and P< 0.05 (t) test.

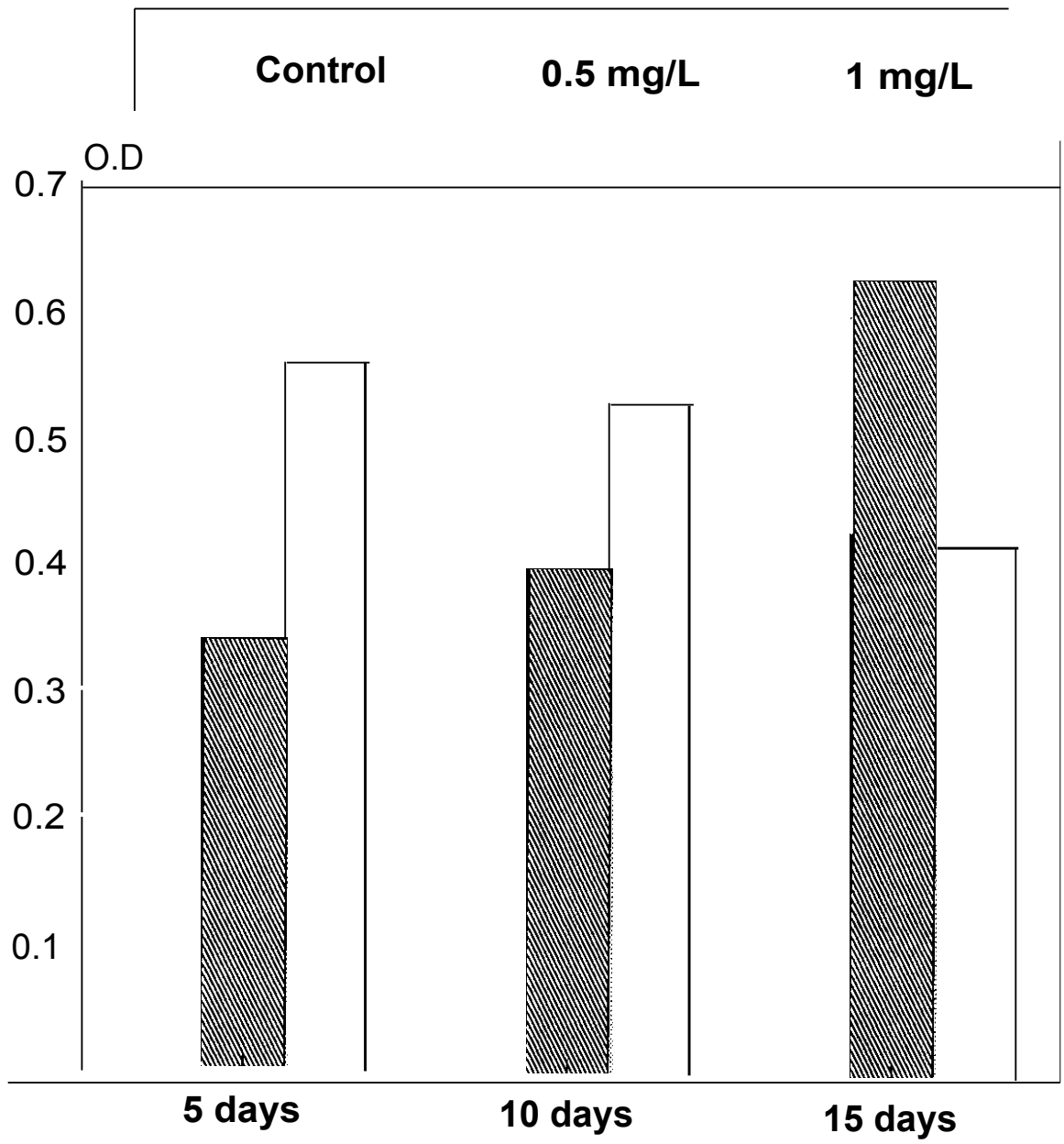


Fig. (66): The mean optical density (O.D.) values of SS group rich protein content in ovary of <u>Orepchromis piloticus</u>

II- Liver :

1- Histological observation :

A section in untreated fish liver (Fig. 67) shows a central vein from which cords of hepatocytes radiate. The hepatocytes have cytoplasmic vacuoles of different sizes. Some hepatocytes are binucleated.

The hepatocyte nucleus is large-centrally located in cells with small vacuoles and small eccentric in cells with large vacuoles.

In fish treated with 0.5 mg/L Sevin for 5 days, most of the cells loose their vacuoles, become collapsed with densly stained nuclei (Fig. 68).

Treatment with 1.0 mg/L Sevin for 5 days, however, resulted in extensive vacuolation of liver cells (Fig.69). After 10 days of treatment with 0.5 mg/L Sevin, (Fig. 70) some hepatocytes retained their vacuolation but most of them were still eosinophillic. Hepatocytes of 1 mg/L Sevin treated fish appeared also vacuolated but to a lesser extent after 10 days (Fig. 71), than 5 days.

After 15 days of treatment with 0.5 mg/L Sevin (Fig. 72), the hepatocytes appeared more vacuolated with signs of degeneration in many of them. In 1 mg/L Sevin treated animal liver, most cells appeared eosinophillic with small pyknotic nuclei (Fig. 73).

Sections in liver of **Oreochromis niloticus** :

Fig. (67) : Control, 1-hepatocytes, 2-kupffer cell, 3-central vein, C: hepatocyte with eosinophillic cytoplasm with small vacuoles and large central nucleus, E: hepatocyte with large vacuole and small eccetric nucleus.

Fig. (68): 0.5 mg/L treated for 5 days, Notice the collapsed eosinophillic hepatocytes and the dilated sinusoids.

Fig. (69):1.0 mg/L treated for 5 days,Notice the extensive vacuolation of hepatocytes.

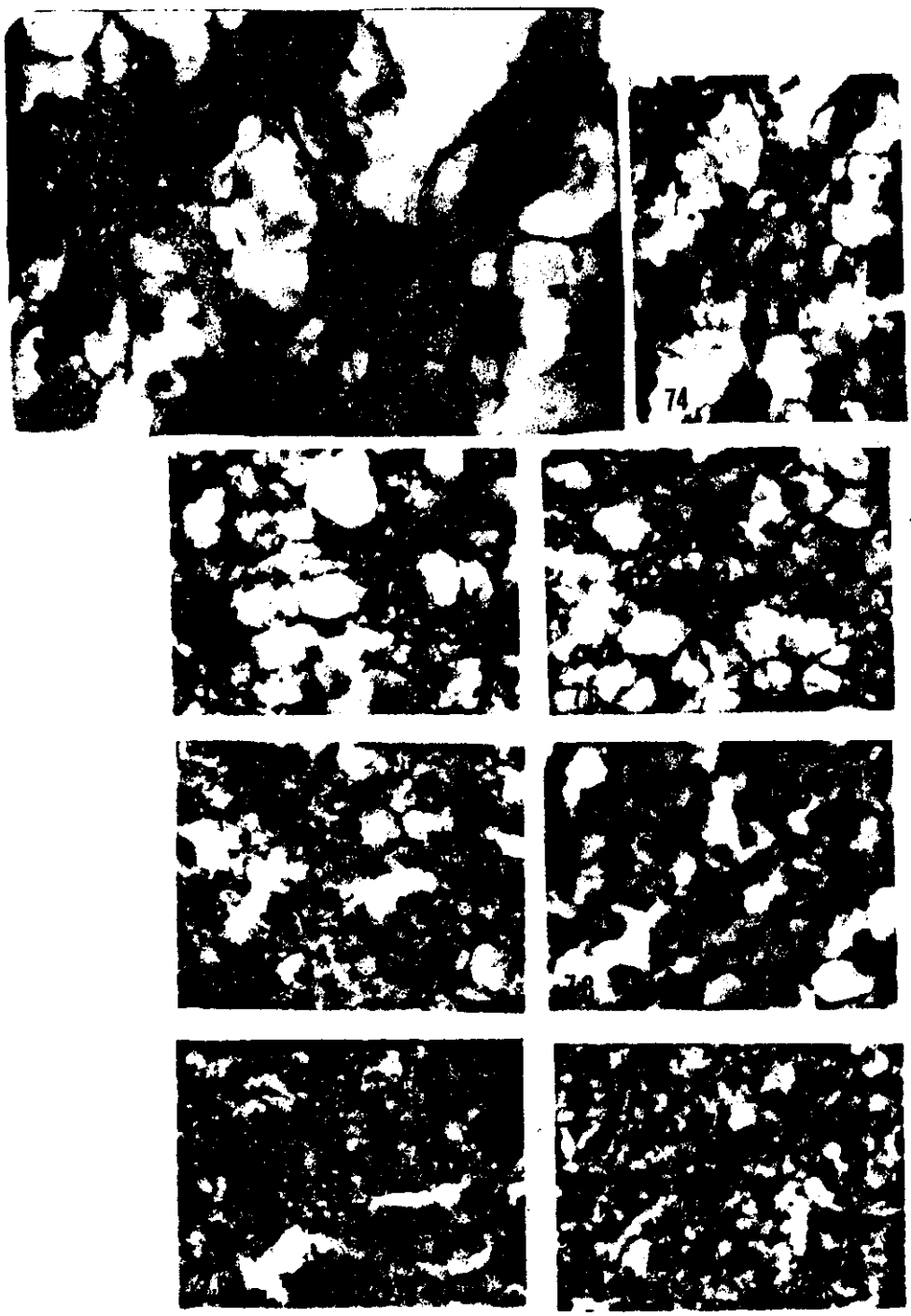
Fig. (70): 0.5 mg/L treated for 10 days.

Fig. (71): 1.0 mg/L treated for 10 days.

Fig. (72): 0.5 mg/L treated for 15 days, d: degeneration.

Fig. (73): 1.0 mg/L treated for '15 days, N: nucleus, V: vacuole, B: blood vessle, SV: small vacuol, K: Kupffer cell.

(H X & E X 500)


74

Sections in liver of **Oreochromis niloticus** .

Fig. (74): Control showing glycogen.

Fig. (75): 0.5 mg/L treated for 5 days.

Fig. (76): 1.0 mg/L treated for 5 days.

Fig. (77): 0.5 mg/L treated for 10 days.

Fig. (78): 1.0 mg/L treated for 10 days.

Fig. (79): 0.5 mg/L treated for 15 days.

Fig. (80): 1.0 mg/L treated for 15 days.

(PAS. X 1000).

Table (8): Effect of Sevin on PAs positive materials in the liver of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean + S.D	0.82 0.02	0.05* 0.03	0.10 0.045
10	Mean ± S.D	0.82 0.02	0.06* 0.01	0.12* 0.05
15	Mean + S.D	0.82 0.02	0.10* 0.01	0.24* 0.127

* Significant difference as compared to control at P< 0.025 and P< 0.05 (t) test.

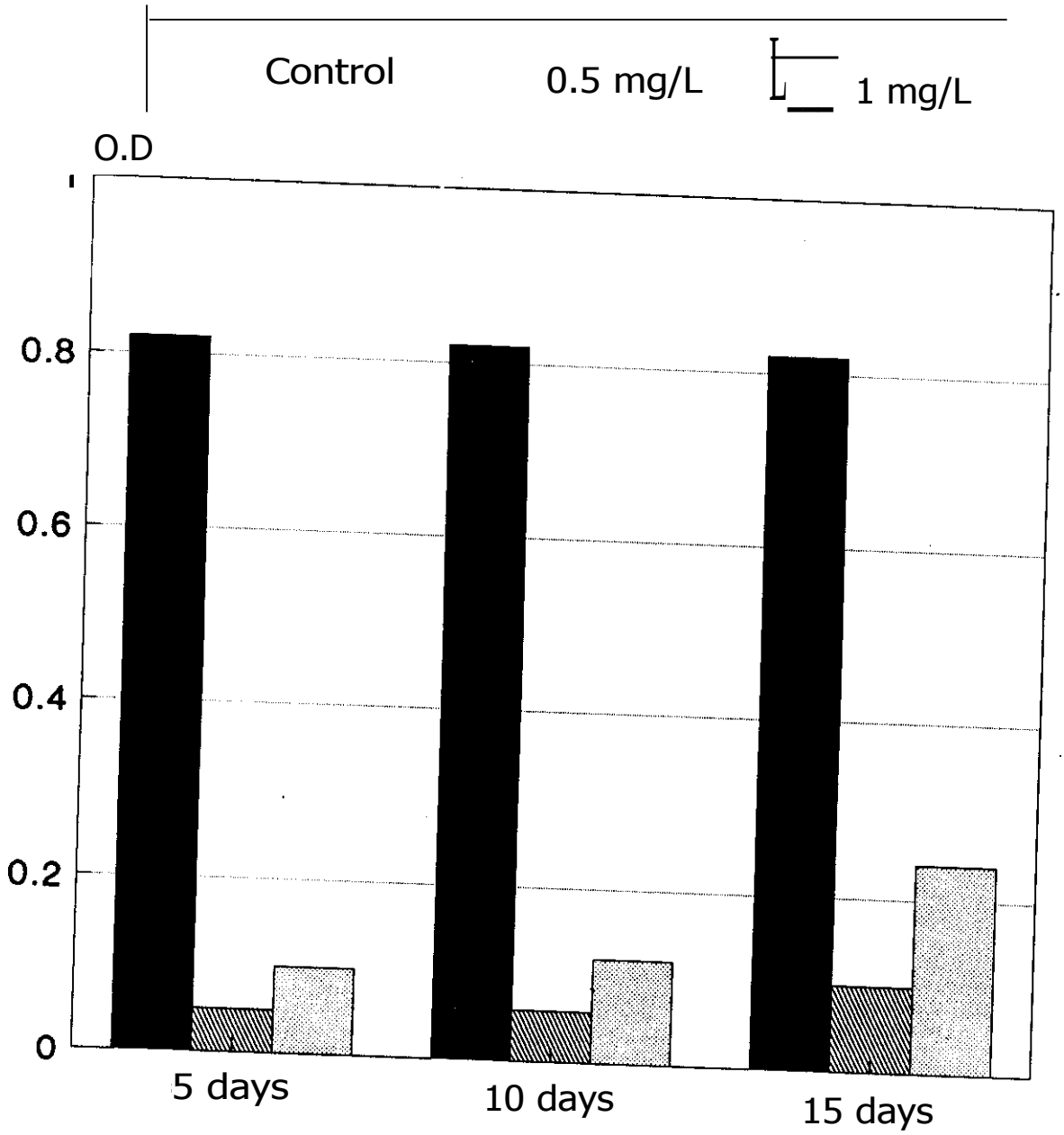
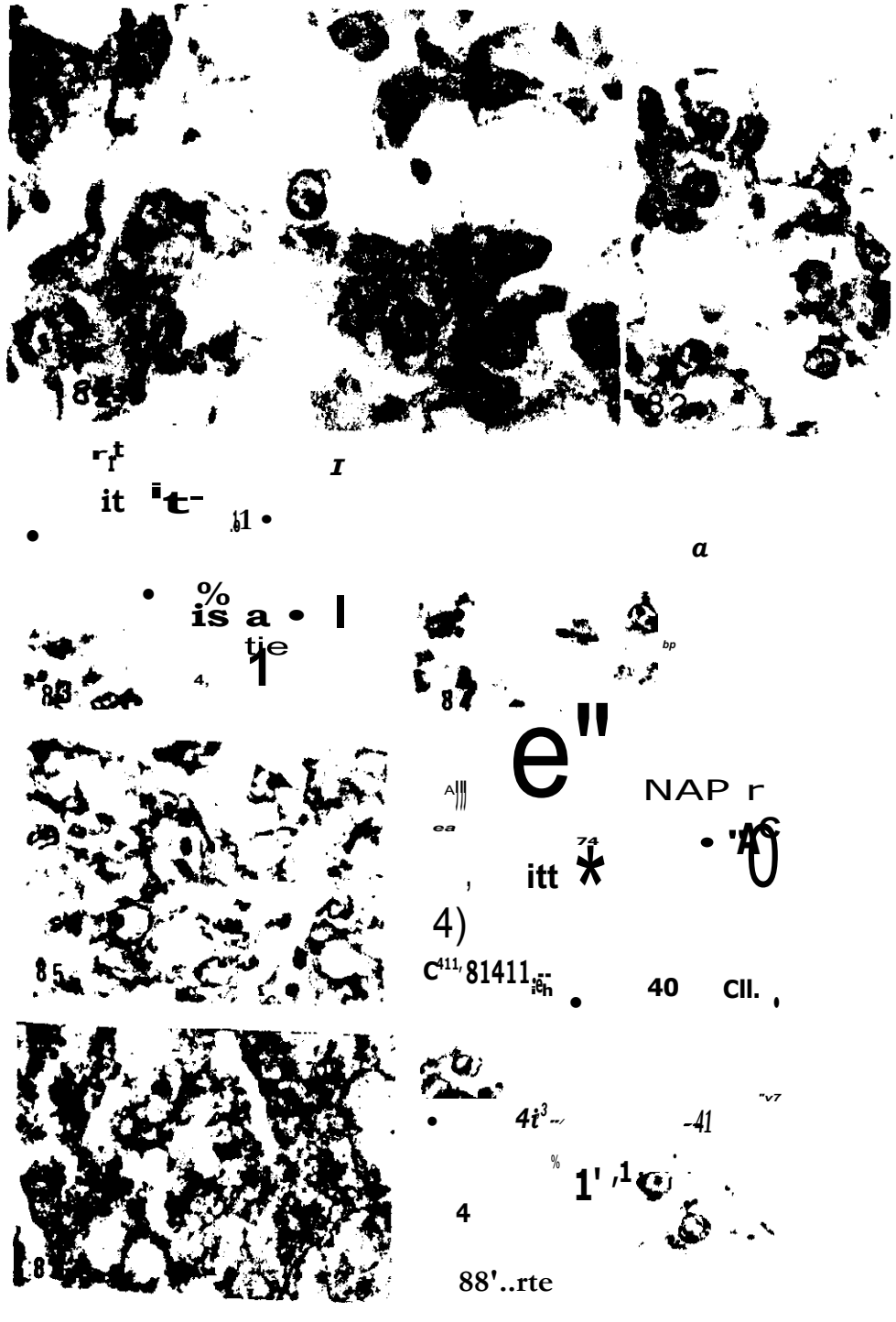


Fig. (81): The mean optical density (O.D.) values of glycogen content (PAS stain) in liver of **Dipay orepchromis s**

RNA content :

Pyronin stainability is demonstrated both in the cytoplasm and nucleus of fish hepatocytes. The nucleolous, however, is very densly stained (Fig. 82). Both nuclear and cytoplasmic stainability are less in hepatocytes of treated animals (Fig. 83-88). In these cells, the cytoplasmic RNA appeared mainly perinuclear (Fig. 86). However, it appeared as large granules in the cytoplasm (Fig. 84).



Sections in liver of **Oreochromis niloticus :**

Fig. (82): Control.

Fig. (83): 0.5 mg/L treated for 5 days.

Fig. (84): 1.0 mg/L treated for 5 days.

Fig. (85): 0.5 mg/L treated for 10 days.

Fig. (86): 1.0 mg/L treated for 10 days.

FIg. (87): 0.5 mg/L treated for 15 days.

Fig. (88): 1.0 mg/L treated for 15 days.

(Methyl green pyronin X 1000).

Cytophotometric measurement of RNA in liver (Table 9, 10, 11 and Fig. 89,89a, 90 & 91) :

The amount of cytoplasmic RNA per cell as demonstrated by pyronin stain was higher in treated animal hepatocytes after 5 and 10 days but less after 15 days of treatment with 0.5 mg/L Sevin as compared to the control groups.

Treatment with 0.5 mg/L Sevin gave the highest optical density value for RNA content after 5 and 10 days (0.398 ± 0.10) and (0.39 ± 0.105) respectively.

Treatment with 1.0 mg/L Sevin comes next to the 0.5 mg/L treatment with values less than those of the control. (0.30 ± 0.07) at 5 days (0.285 ± 0.14) at (10 days and higher than that of the control (0.44 ± 0.15) at 15 days, where the value of the control was (0.37+ 0.12) throughout the period of the experiment.The statistical significance from control was, however,for the values of 1 mg/L treated animals only.

Although the absolute values for cytoplasmic RNA content is statistically significant from control in 1.0 mg/L treated fish only, the amount of cytoplasmic RNA/DNA is difinitely less than that of the control at 5 and 10 days for both concentrations.

Table (9): Effect of Sevin on RNA content in liver *O. **niloticus*** (Methyl green pyronin).

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean	0.37	0.398	0.30*
	± S.D	0.12	0.10	0.07
10	Mean	0.37	0.39	0.285*
	± S.D	0.12	0.105	0.14
15	Mean	0.37	0.34	0.44*
	± S.D	0.12	0.10	0.15

* Significant difference as compared to control at $P< 0.025$ and $P< 0.05$ (t) test.

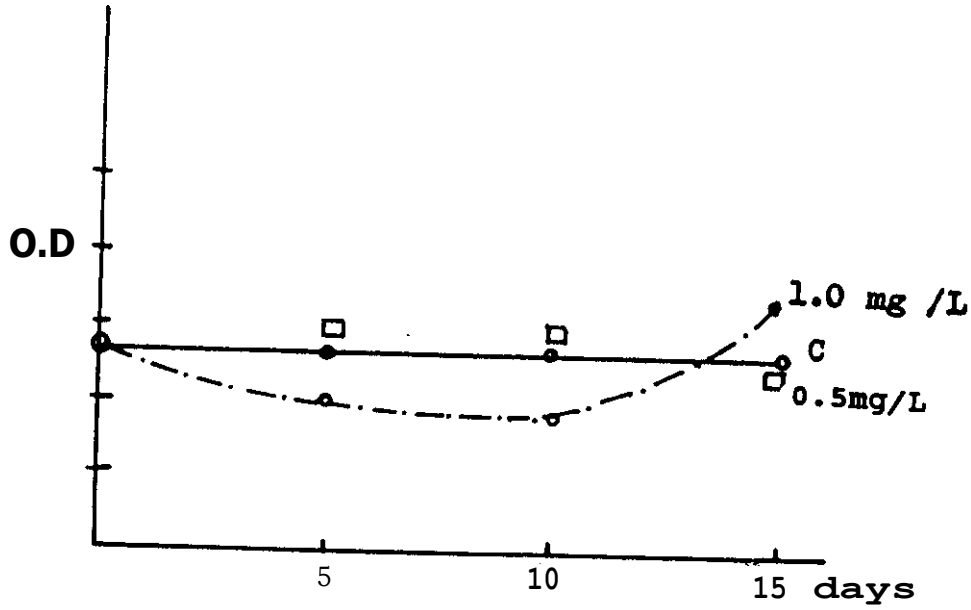


Fig. (89): Changes in cytoplasmic RNA content of hepatocytes of fish treated with. 0.5 and 1.0 mg/L sevin for 5,10 and 15 days.

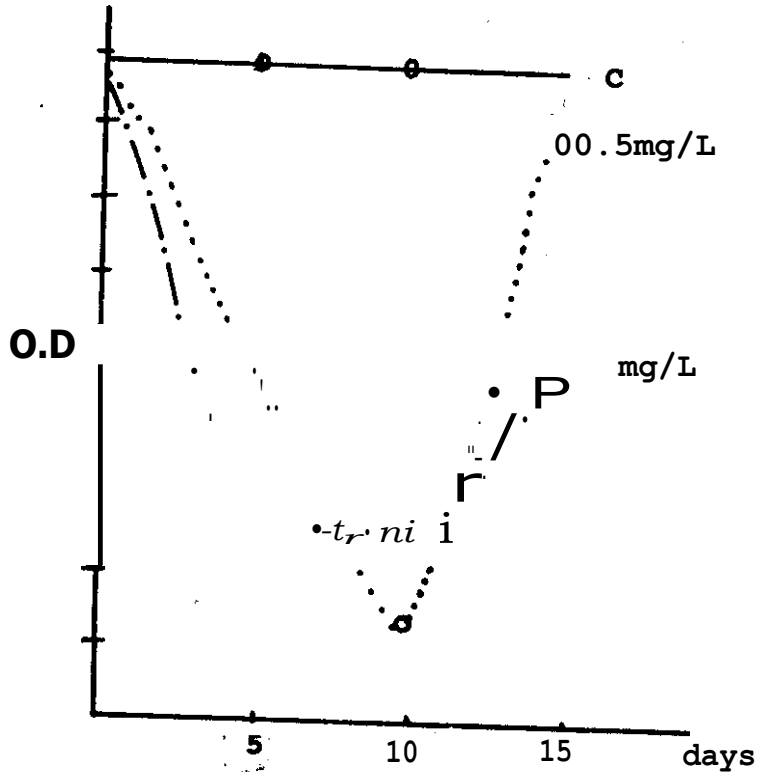


Fig. (89a): Relative amount of cytoplasmic RNA per DNA in hepatocytes of fish treated with 0.5 and 1.0 mg/L sevin for 5,10 and 15 days.

Table (10): Effect of Sevin on amount of cytoplasmic RNA/DNA in hepatocytes of fish.

Days	Control	0.5 mg/L	1.0 mg/L
5	2.18	1.33	1.07
10	2.18	0.68	0.93
15	2.18	1.988	1.375

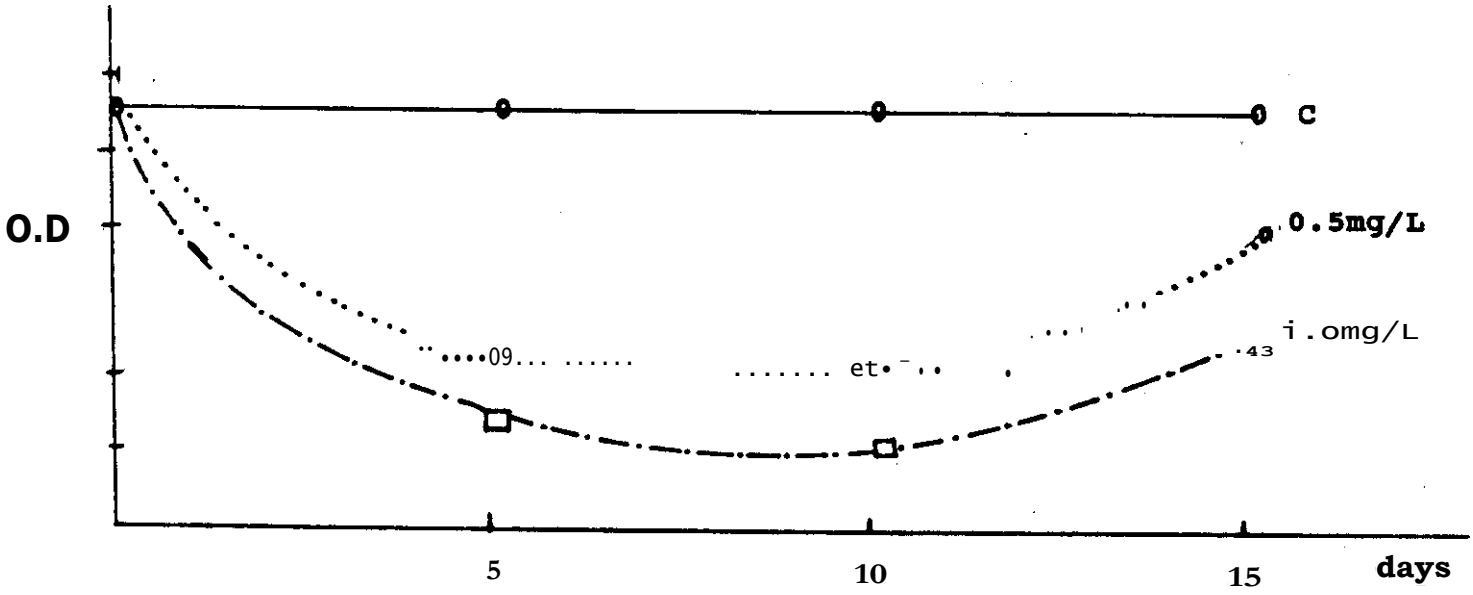


Fig. (90): Relative O.D values for nuclear RNA per hepatocytes of fish treated with 0.5 and 1.0 mg/L sevin for 5,10 and 15 days.

Table (11): Effect of Sevin on nuclear RNA/DNA of hepatocytes of fish.

Days	Control	0.5 mg/L	1.0 mg/L
5	5.47	2.53	2.57
10	5.47	2.87	2.28
15	5.47	4.96	2.44

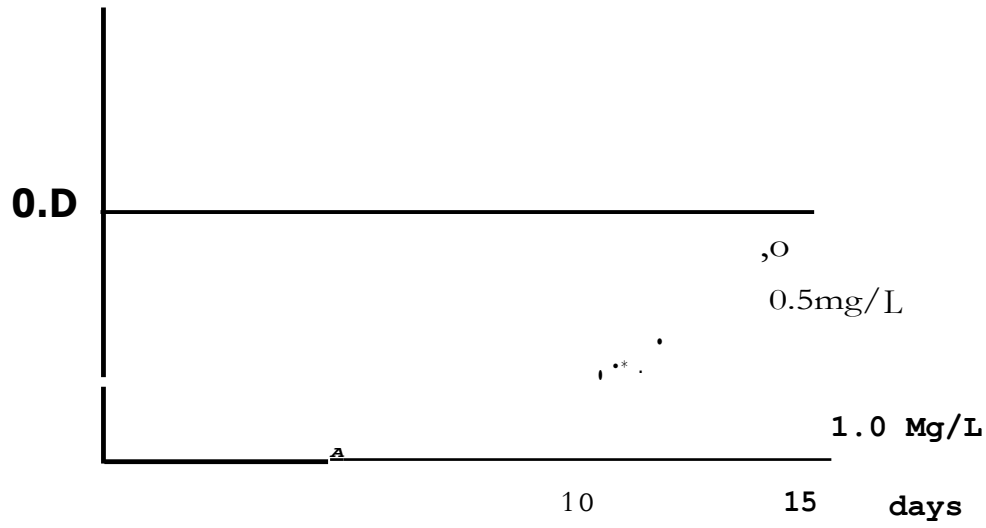


Fig. (91): Relative change in nuclear RNA/DNA of hepatooytes of fish treated with 0.5 and 1.0 mg/L sevin for 5,10 and 15 days.

Cytophotometric measurement of DNA (Feulgen) in liver (table 12, Fig. 95,96) :

The amount of DNA per nucleus was measured in sections stained by Feulgen stain (Fig. 92,93,94). From values obtained, the mean optical density values revealed that treatment with the insecticide resulted in an increase of nuclear stainability by Feulgen.The increment was directly proportional to the dose of the insecticide but inversely proportional to the period the fish spent in the insecticide polluted water.

The mean O.D. values for nuclear Feulgen stainability for the control animals was (0.17 ± 0.048) for all the samples obtained through out the period of the experiment.

For the fish incubated in 0.5 mg/L carbamate polluted water, the O.D. values were (0.30 ± 0.075) after 5 days, (0.26+0.45) after 10 days and (0.17+0.048) after 15 days. For animals incubated in 1.0 mg/L carbamate polluted water, the O.D. values were (0.28 ± 0.065) after 5 days, (0.30 ± 0.043) after 10 days and (0.32 ± 0.084) after 15 days.

Statistical analysis of the above data indicated that, the difference in DNA content of 0.5 and 1.0 mg/L treated animals was insignificant while both were significantly higher than the control.

From the analysis of frequency distribution curves, it may be indicated that treatment with the insecticides resulted in accumulation of high amounts of nuclear DNA which recessed by time to become almost similar to control after 15 days of treatment with 0.5 mg/L. However, treatment with 1.0 mg/L resulted in persisting high nuclear DNA values even after 15 days of incubation.

Sections in liver of **Oreochromis niloticus** .

Fig. (92): Control showing nuclear DNA content.

Fig. (93): 0.5 mg/L treated for 10 days.

Fig. (94): 1.0 mg/L treated for 10 days

(Feulgen X 1000).

Table (12): Effect of Sevin on DNA content in liver of 0. niloticus (Fulgen).

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.17 0.0484	0.30 0.075	0.28 0.065
10	Mean ± S.D	0.17 0.0484	0.26 0.045	0.30 0.043
15	Mean ± S.D	0.17 0.0484	0.17 0.048	0.32 0.084

Significant differences as compared to control at $P < 0.025$ and $P < 0.05$ (t) test.

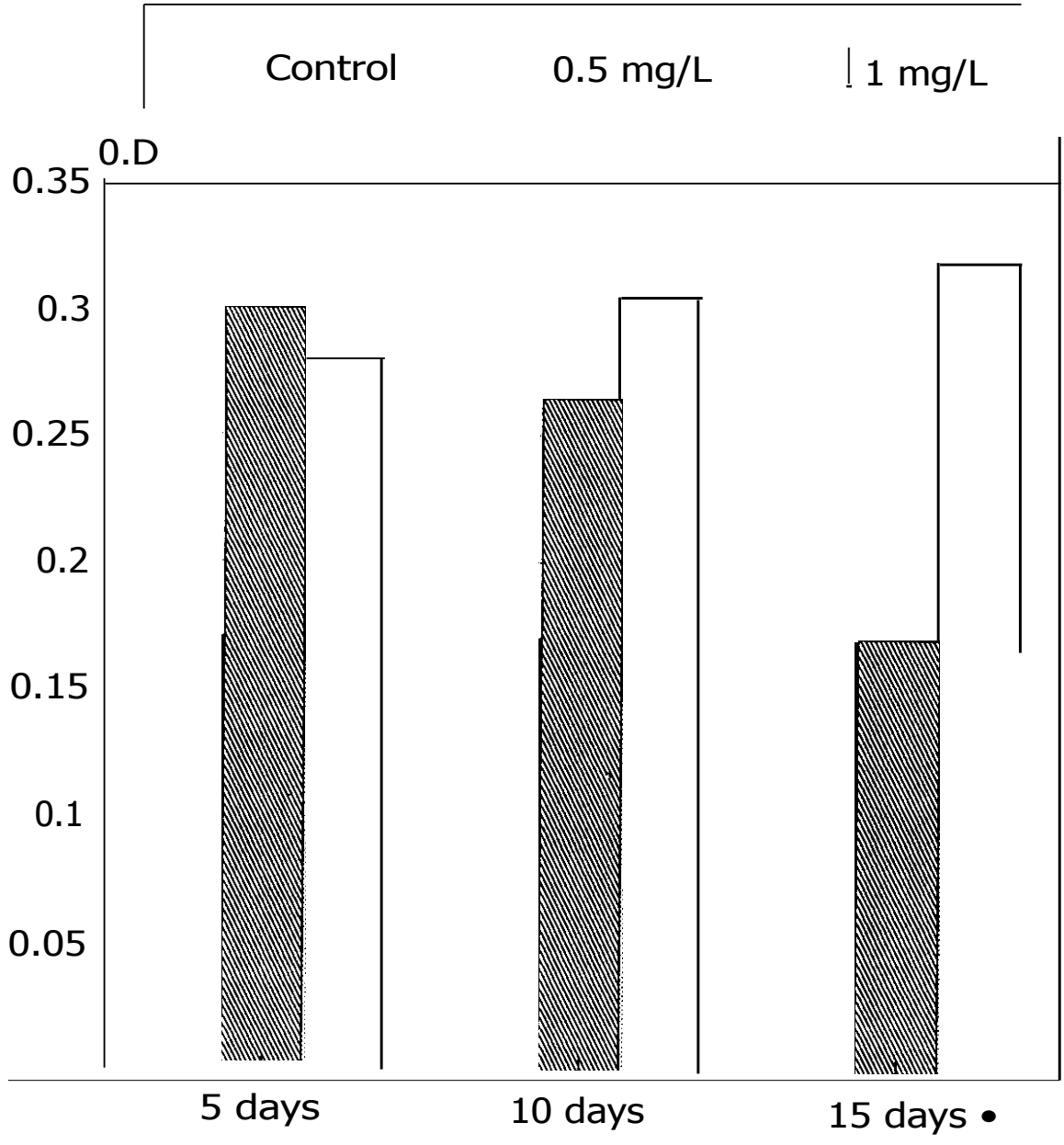
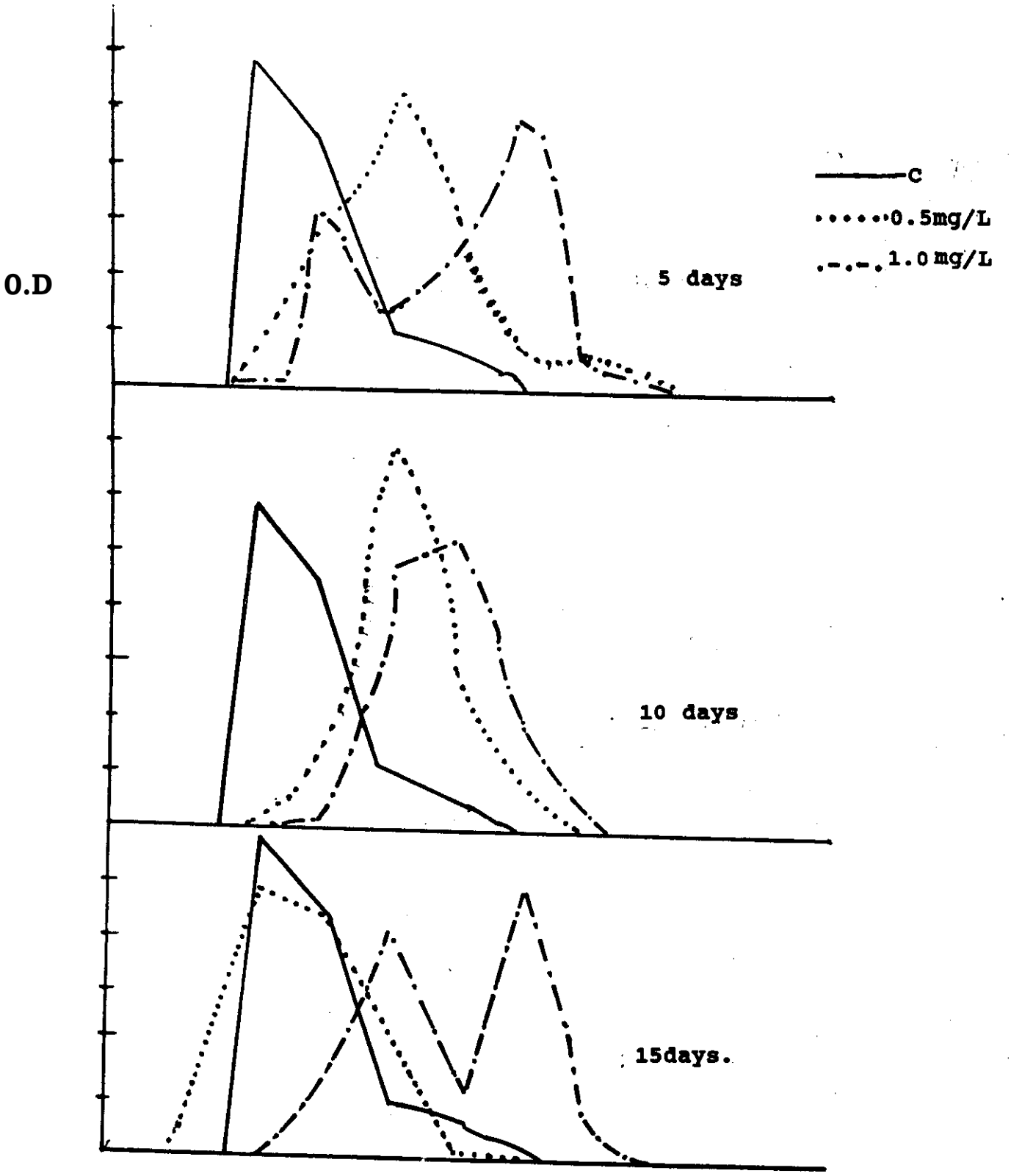


Fig. (95): The mean optical density values of DNA .(Feulgen stain) content (PAS, stain) in liver of **Oreochromiq niloticus**

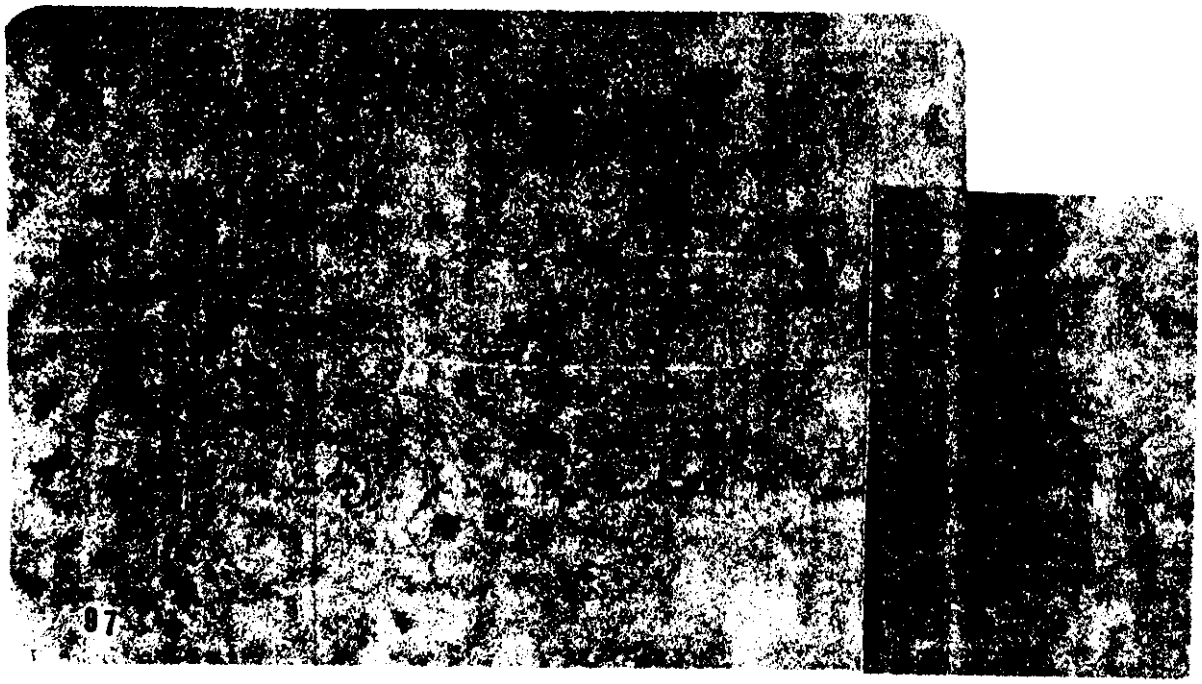


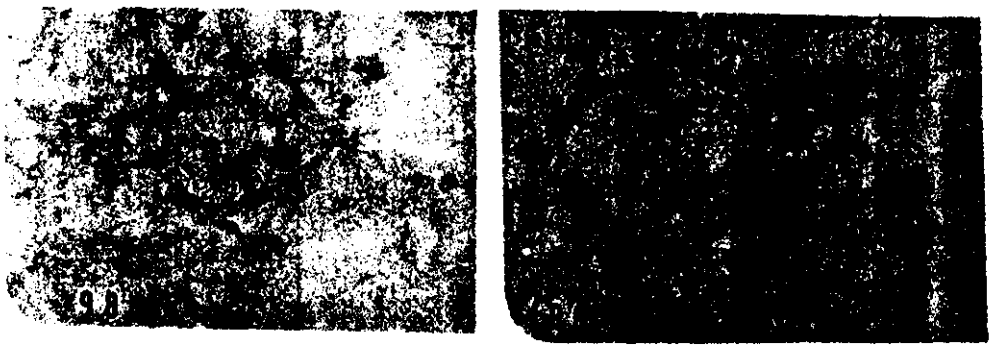
Wig. (96): The mean 0.D of DNA content (Feulgen stain) of hepatocytes of fishes treated with 0.5 and 1.0 mg/L sevin for 5,10 and 15 days

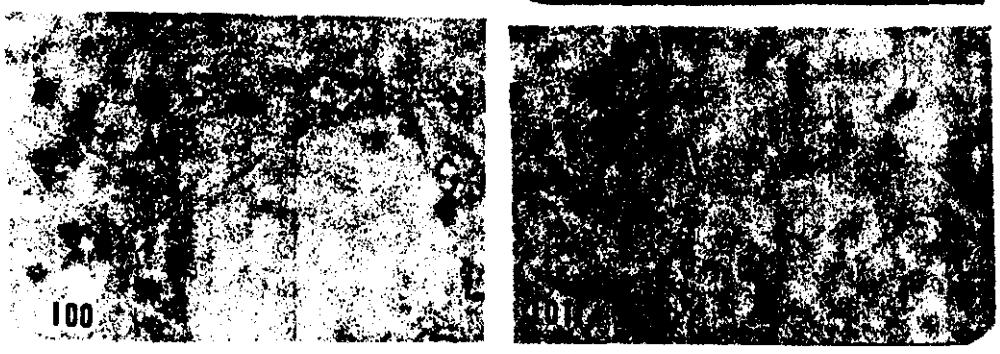
Proteins in liver of treated fish :

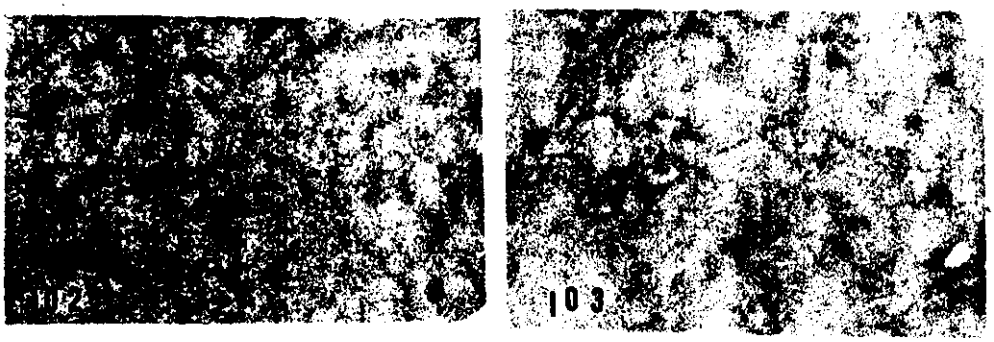
Proteins rich in sulfer containing amino acids appear mainly in the cytoplasm of hepatocytes (Fig. 103). More stainable proteins of this kind are found around the nucleus and in the nucleolar area inside the nucleus. The nucleus of RBCs is highly rich in these proteins.

Basic proteins rich in NH_2 group containing amino acids are found both in the cytoplasm and hepatocyte nuclei (Fig.105).The nuclei of the control group, however, are highly stained for these proteins than treated animal hepatocytes nuclei (Fig. 105-111).


97


98


100
101


102
103

Sections in liver of **Oreochromis niloticus :**

Fig. (97): Control, showing keratin content.

Fig. (98): 0.5 mg/L treated for 5 days.

Fig. (99): 1.0 mg/L treated for 5 days.

Fig. (100): 0.5 mg/L treated for 10 days.

Fig. (101): 1.0 mg/L treated for 10 days.

Fig. (102): 0.5 mg/L treated for 15 days.

Fig. (103): 1.0 mg/L treated for 15 days.

(Performic acid-schiff X 1000).

Cytophotometric measurement of keratin in liver : Keratin-like proteins (Table 13, Fig. 104):

The amount of keratin per cell as demonstrated by performic acid Schiff's stain generally decreased in treated animal hepatocytes.as compared to control.

Treatment with 0.5 mg/L Sevin resulted in highest optical density values relative to keratin content after 5 days (0.54 ± 0.05), 10 days (0.498 ± 0.12), and 15 days (0.57 ± 0.035).

Treatment with 1.0 mg/L comes next to the first treatment with value of (0.54 ± 0.125) at 5 days, (0.54 ± 0.08) at 10 days and (0.26 ± 0.11) at 15 days as compared to control for which the value was (0.61 ± 0.11).

There is a statistically significant difference at 0.5 mg/L after 5 and 10 days as compared with the control. Also there is a highly significant difference after treatment with 1.0 mg/L for 15 days as compared with control groups.

Table (13): Effect of Sevin on keratin content in liver of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.60 0.11	0.54* 0.05	0.54 0.125
10	Mean ± S.D	0.60 0.11	0.498* 0.12	0.54 0.78
15	Mean ± S.D	0.60 0.11	0.57 0.035	0.26* 0.11

* Significant difference as compared to control at $P < 0.025$ and $P < 0.05$ (t) test.

10 5

1 06

1 G 7

10 8 ,

40.

109

110

111

Sections in liver of **Oreochromis niloticus** :

Fig. (105): Control, showing amino group rich proteins.

Fig. (106): 0.5 mg/L treated for 5 days.

Fig. (107): 1.0 mg/L treated for 5 days.

Fig. (108): 0.5 mg/L treated for 10 days.

Fig. (109): 1.0 mg/L treated for 10 days.

Fig. (110): 0.5 mg/L treated for 15 days.

Fig. (111): 1.0 mg/L treated for 15 days.

(Ninhydrin Schiff X 1000).

Cytophotometric measurement of amino group rich proteins in liver (Table 14, Fig. 112) :

The amount of amino group in liver tissue demonstrated by ninhydrin Schiff stain is higher in treated animal hepatocytes than that of the control. It is highly statistically significant after 5 days of treatment with 1.0 mg/L Sevin (0.89+0.20) as compared to control (0.48+0.14). However, the difference was insignificant after 5 days of treatment with 0.5 mg/L (0.47 ± 0.11), after 10 days with 0.5 mg/L (0.44+0.08), after 15 days with 0.5 mg/L (0.46+ 0.06) and after 15 days with 1.0 mg/L Sevin (0.46+0.06)and after 15 days with 1.0 mg/L Sevin (0.46+ 0.13) as compared to the control (0.48+0.14). It was statistically significant after 10 days treatment with 1.0mg/L Sevin (0.37+0.07) as compared to the control (0.48 ± 0.14).

Table (14): Effect of Sevin on Amino group rich proteins content in liver of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.48 0.14	0.47 0.11	0.89* 0.20
10	Mean ± S.D	0.48 0.14	0.44 0.08	0.37* 0.07
15	Mean ± S.D	0.48 0.14	0.46 0.06	0.46 0.13

* Significant difference as compared to control at P < 0.025 and P < 0.05 (t) test.

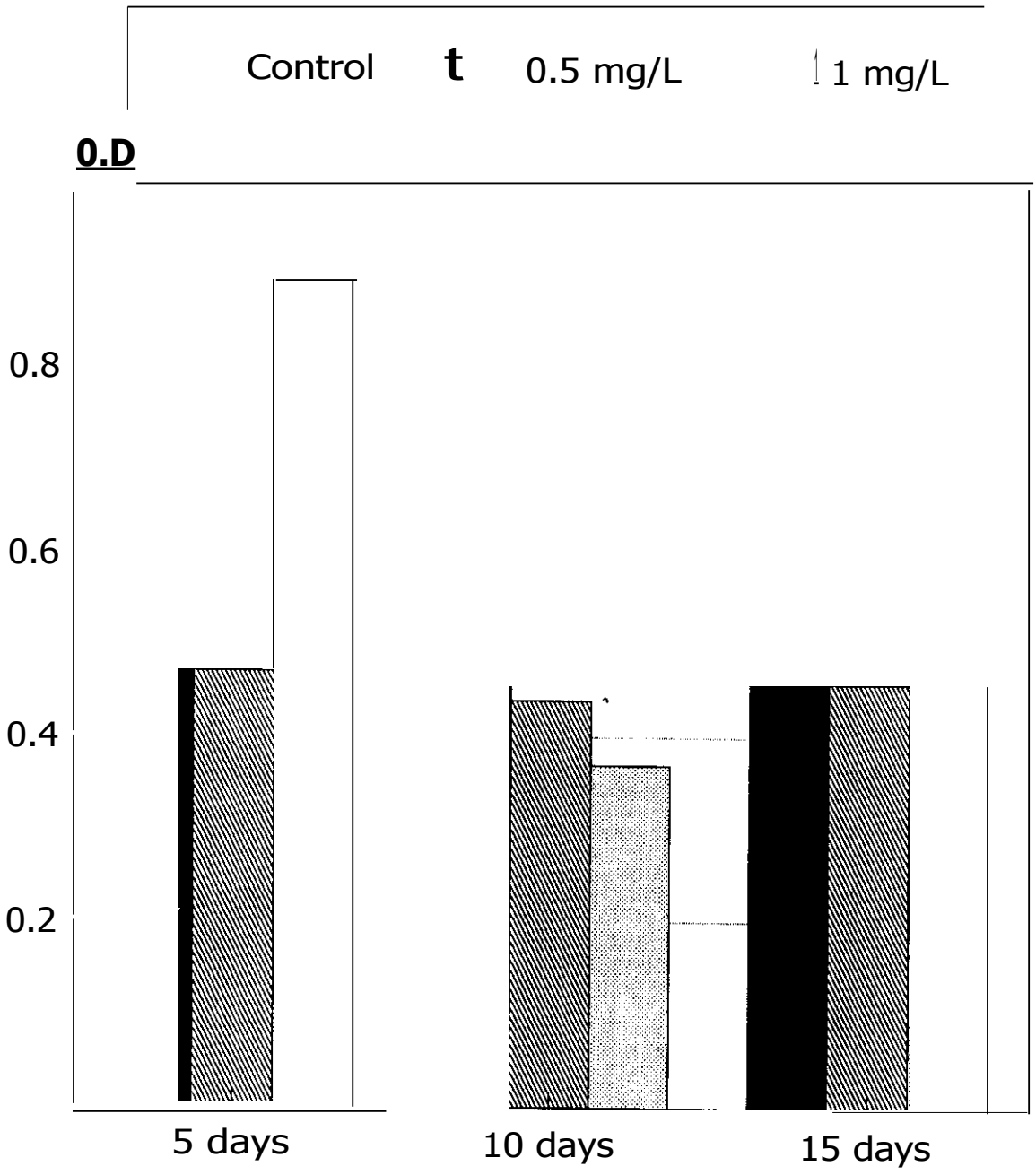


Fig. (112): The mean optical density values of Amino group rich profein content in liver of **Oreochromis niloticus•**

Cytophotometry of proteins rich in SS group in liver (Table 15, Fig. 113) :

The amount of proteins rich in disulfied containing amino acids per cell as demonstrated by performic acid alcian blue stain was significantly higher in treated animal hepatocytes as compared to control. The optical density values obtained after treatment with 0.5 mg/L Sevin were generally higher than control after 5 days (0.08 ± 0.03), 10 days (0.155 ± 0.03), and 15 days (0.12 ± 0.04) of treatment.

Treatment with 1.0 mg/L was also higher than control with value of (0.11 ± 0.07) at 5 days, (0.06 ± 0.01) at 10 days and (0.14 ± 0.04) at 15 days. The value obtained for control was (0.02+0.01).The highest affect was represented at 10 days with 0.5 mg/L and at 15 days with 1.0 mg/L.

Table (15): Effect of Sevin on SS. group rich proteins content in liver of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.02 0.01	0.08* 0.03	0.11* 0.07
10	Mean ± S.D	0.02 0.01	0.155* 0.03	0.06* 0.01
15	Mean ± S.D	0.02 0.01	0.12* 0.04	0.14* 0.04

* Significant difference as compared to control at $P < 0.025$ and $P < 0.05$ (t) test.

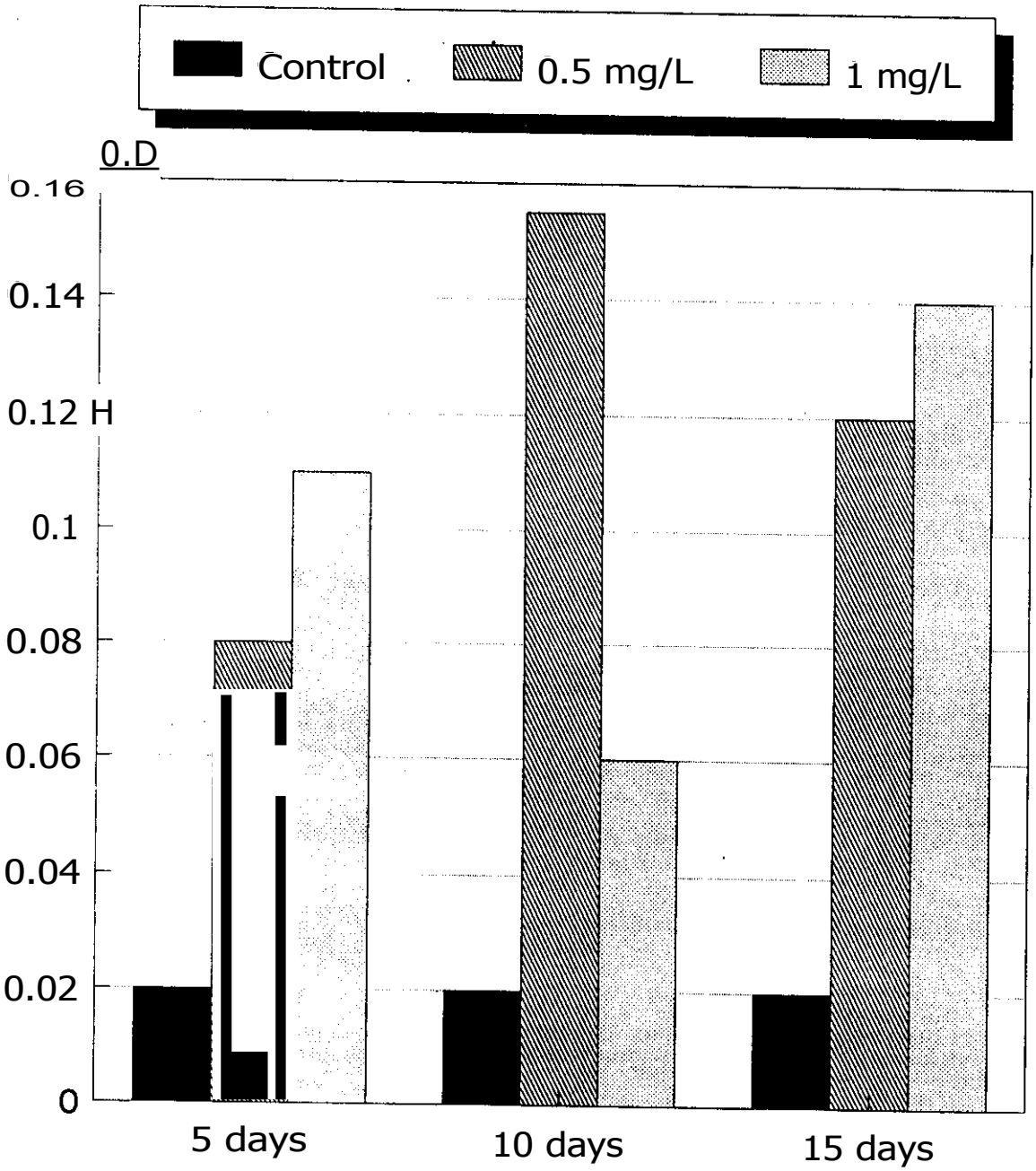


Fig. (113): The mean optical density values of SS group content in liver of **Oreochromis niloticus.**

adjacent to fibres showing signs of coagulative degeneration (Fig. 117). After 10 days of treatment (Fig. 119) greater flocculation, vacuolation and coagulation is observed in most of the muscle fibres. After 15 days of treatment, clear signs of dystrophy and fatty infiltration are evident in some parts of the muscle (Fig. 121). In other parts, vacuolation and complete coagulative necrosis with fatty infiltration and hypertrophy of muscle fibres are observed (Fig. 122).

Longtudinal sections in muscle of **Oreochromis nilotious** :

Fig. (114): Control.

Fig. (115): 0.5 mg/L treated for 5 days.

Fig. (116): 1.0 mg/L treated for 5 days.

Fig. (117): 1.0 mg/L treated for 5 days.

Fig. (118): 0.5 mg/L treated for 10 days.

Fig. (119): 1.0 mg/L treated for 10 days.

Fig. (120): 0.5 mg/L treated for 15 days.

Fig. (121): 1.0 mg/L treated for 15 days.

Fig. (122): 1.0 mg/L treated for 15 days

(H X & E X 400).

Cholinesterase activity :

In control fish (Fig. 123), all the muscle fibres are innervated.

In fish treated with 0.5 mg/L Sevin for 3 days, (Fig. 124) the fibers which still have their nerve supply appear normal while those without clearly active nerve supply appear pale, structureless and dystrophied. After 5 days of treatment more fibres appear denervated and loosing their striation (Fig.126).These changes are more prominant after 10 days (Fig.128) and 15 *days* of treatment (Fig.130)

In 1.0 mg/L treated fish, a larger number of denervated fibres appear after 3 days of treatment (Fig. 125) compared with 0.5 mg/L treated. Such denervation and loss of fibre structure increase after 5 days (Fig. 127), 10 days (Fig. 129) to became very clear after 15 days (Fig. 131).

Longtudinal sections in muscule of **Oreochromis niloticus**

Fig. (123): Control.

Fig. (124): 0.5 mg/L treated for 3 days.

Fig. (125): 1.0 mg/L treated for 3 days.

Fig. (126): 0.5 mg/L treated for 5 days.

Fig. (127): 1.0 mg/L treated for 5 days.

Fig. (128): 0.5 mg/L treated for 10 days.

Fig. (129): 1.0 mg/L treated for 10 days.

Fig. (130): 0.5 mg/L treated for 15 days.

Fig. (131): 1.0 mg/L treated for 15 days.

(Acetylcholin stain X 400).

Cytophotmetric measurement of glycogen in muscle (table 16, Pig. 132) :

The amount of PAS positive material is generally patchy high in the periphery treated animal muscle fibres than that of the control especially at 5 days after treatment. The optical density value relative to glycogen content obtained after 0.5 mg/L Sevin treatment for 5 days was (1.32 ± 0.12), for 10 days (1.22 ± 0.10) and 15 days (1.24 ± 0.11).

Treatment with 1.0 mg/L gave values of (1.38 ± 0.07) at 5 days (1.37 ± 0.08) at 10 days and (1.25 ± 0.08) at 15 days respectively as compared to the control for which the value was (1.23 ± 0.13).

Table (16): Effect of Sevin on PAs positive materials in muscle of 0. niloti us.

Days		Control	Treatment 0.5 mg/L	1.0 mg/L
5	Mean	1.23	1.32*	1.38*
	+ S.D	0.13	0.12	0.07
10	Mean	1.23	1.22	1.37*
	± S.D	0.13	0.10	0.08
15	Mean	1.23	1.24	1.25
	± S.D	0.13	0. 11	0.08

* Significant difference as compared to control at P < 0.025 and P < 0.05 (t) test.

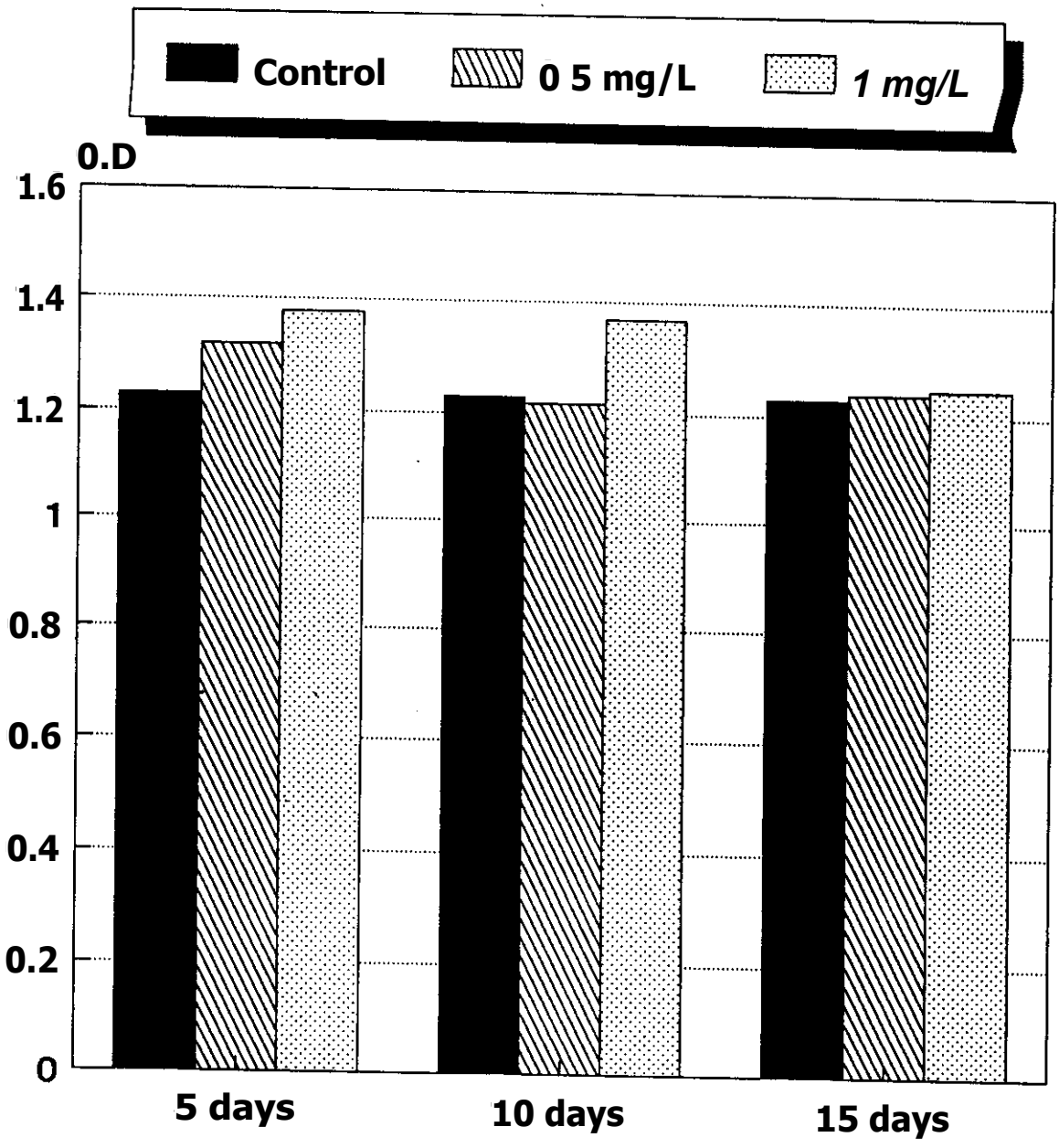


Fig. (132):. The mean optical density values of glycogen content in muscle of Oreochromi§ niloticu§-

Longtudinal sections in muscle of **Oreochromis niloticus** .

Fig. (133): Control, showing the glycogen content.

Fig. (134): 0.5 mg/L treated for 5 days.

Fig. (135): 1.0 mg/L treated for 5 days.

Fig. (136): 0.5 mg/L treated for 10 days.

Fig. (137): 1.0 mg/L treated for 10 days.

Fig. (138): 0.5 mg/L treated for 15 days.

Fig. (139): 1.0 mg/L treated for 15 days.

(PAS-Methylgreen. X 1000).

Table (17): Effect of Sevin on RNA content in muscle of O. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean ± S.D	0.41 0.07	0.47 0.16	0.24* 0.08
10	Mean ± S.D	0.41 0.07	0.71* 0.08	0.36* 0.07
15	Mean ± S.D	0.41 0.07	0.68* 0.06	0.41 0.09

* Significant difference as compared to control at $P < 0.025$ and $P < 0.05$ (t) test.

Cytophotmetric measurement of RNA in muscle (table 17 Fig. 140) :

The relative amount of RNA as demonstrated by pyronin stain is generally higher in 0.5mg/L treated animal muscle fibres at 5 days (0.47 ± 0.16), 10 days (0.71+0.08) and 15 days (0.68 ± 0.06) than control (0.41 ± 0.07).

However, treatment with 1.0 mg/L in generally lower than the relative amount for control for all periods of treatment. It was (0.24 ± 0.08) at 5 days and (0.36+0.07) at 10 days and equal to the control at 15 days (0.41+0.09)

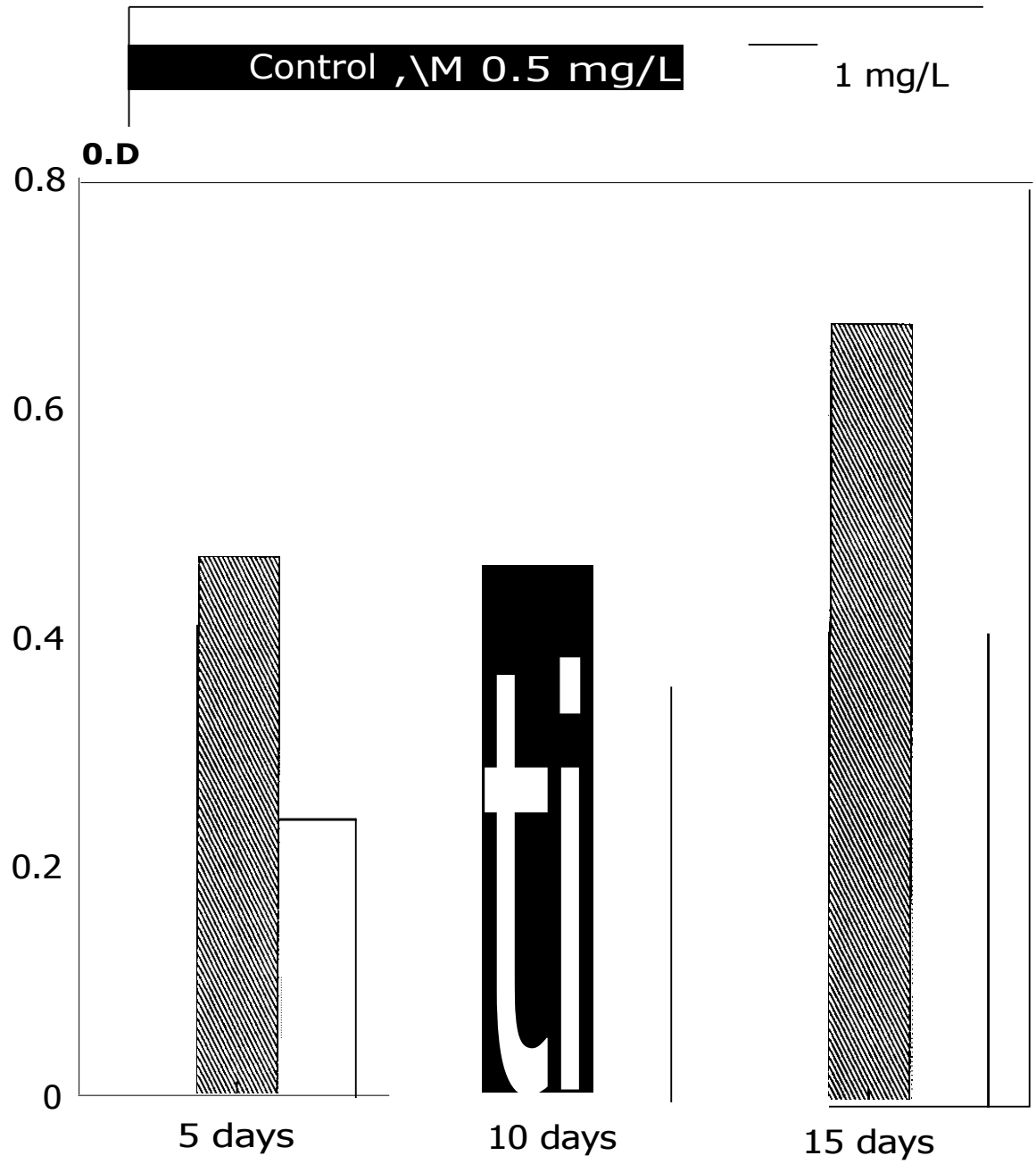


Fig.(140): The mean optical density values of RNA content in muscle of **Oreochromis niloticus**.

Cytophotometric measurement of keratin in muscle (Table 18, Fig. 141):

The amount of keratin per cell as demonstrated by performic acid Schiff stain is generally higher in treated animal muscle fibres as compared to control.Treatment with 0.5 mg/L Sevin resulted in the highest value of optical density relative to keratin content after 5 days (0.41 ± 0.08), 10 days (0.84 ± 0.18), and 15 days (0.54 ± 0.09).

Treatment with 1.0 mg/L came next to the first treatment with values of (0.38+0.07) at 5 days, (0.59+0.17) at 10 days, and (0.49+0.06) at 15 days as compared to control for which the values was (0.30 ± 0.44).

Table (18): Effect of Sevin on keratin content in muscle of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean	0.30	0.41	0.38
	± S.D	0.44	0.08	0.07
10	Mean	0.30	0.84	0.59
	± S.D	0.44	0.18	0.17
15	Mean	0.30	0.54	0.49
	± S.D	0.44	0.09	0.06

* Significant difference as compared to control at P < 0.025 and P < 0.05 (t) test.

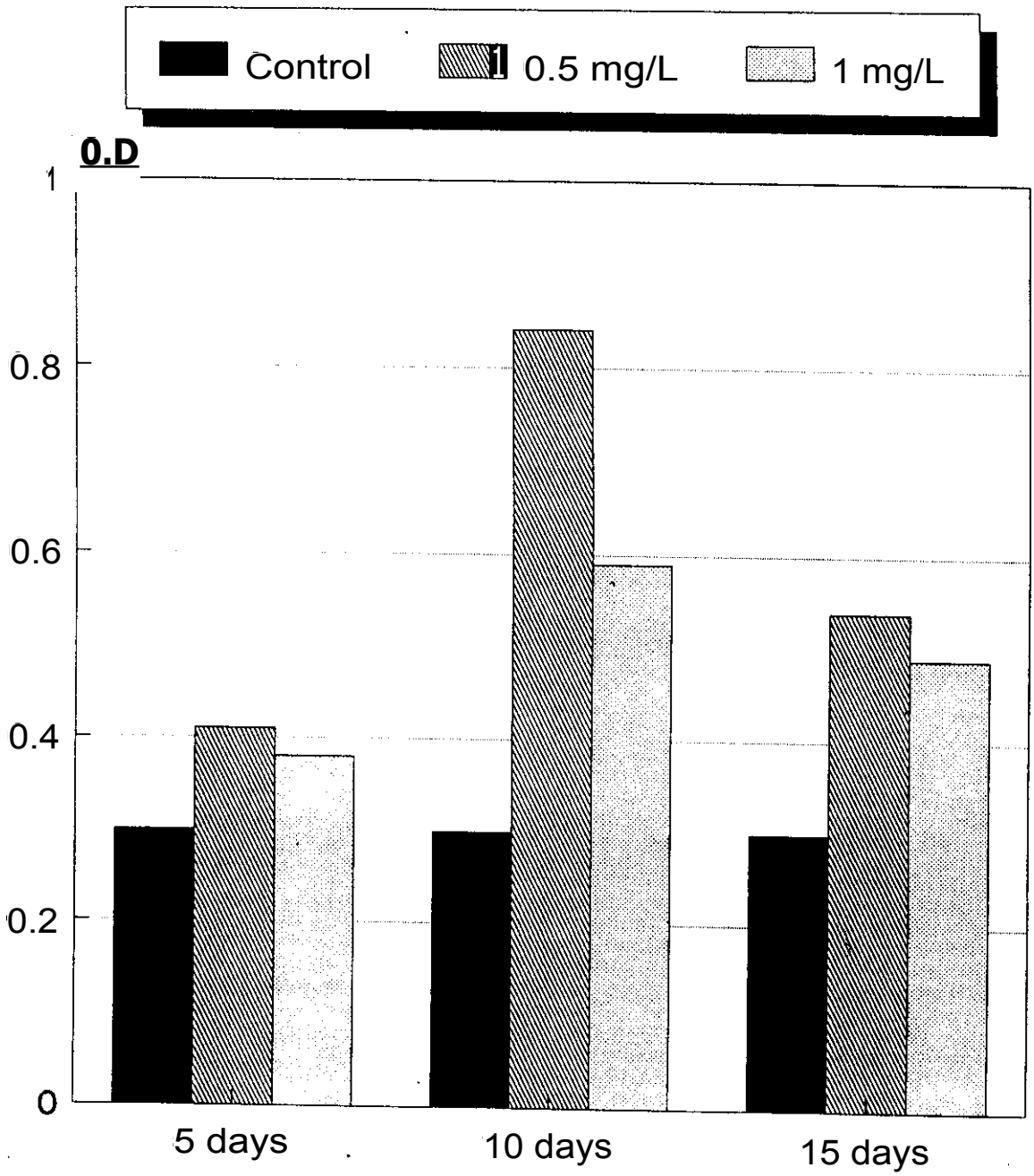


Fig.(141): The mean optical density values of keratin content in muscle of **Oreochromis niloticus•**

Cytophotometric measurement of amino group rich protein in muscle (table 19, Fig. 142) :

The O.D. relative to the amount of amino group rich proteins per cell as• demonstrated by Ninhydrin Schiff stain is generally high in treated animal muscle fibres than that of control. Treatment with 0.5 mg/L Sevin resulted in the highest optical density values which were (1.79 ±0.20) after 5 days, (1.81+0.22) after 10 days (1.79+0.18) after 15 days of treatment compared to (1.33 ± 0.24) for control.

Treatment with 1.0 mg/L comes next to the first treatment with value of (1.69+0.25) at 5 days (1.83+0.23) at 10 days and (1.84 ± 0.32) at 15 days as compared to control for which the value was (1.33 ± 0.24).

Table (19): Effect of Sevin on amino group content in muscle of 0. niloticus.

Days		Control	Treatment	
			0.5 mg/L	1.0 mg/L
5	Mean	1.33	1.79*	1.69*
	± S.D	0.24	0.20	0.25
10	Mean	1.33	1.81*	1.83*
	± S.D	0.24	0.22	0.23
15	Mean	1.33	1.79*	1.84*
	± S.D	0.24	0.18	0.32

* Significant difference as compared to control at $P < 0.025$ and $P < 0.05$ (t) test.

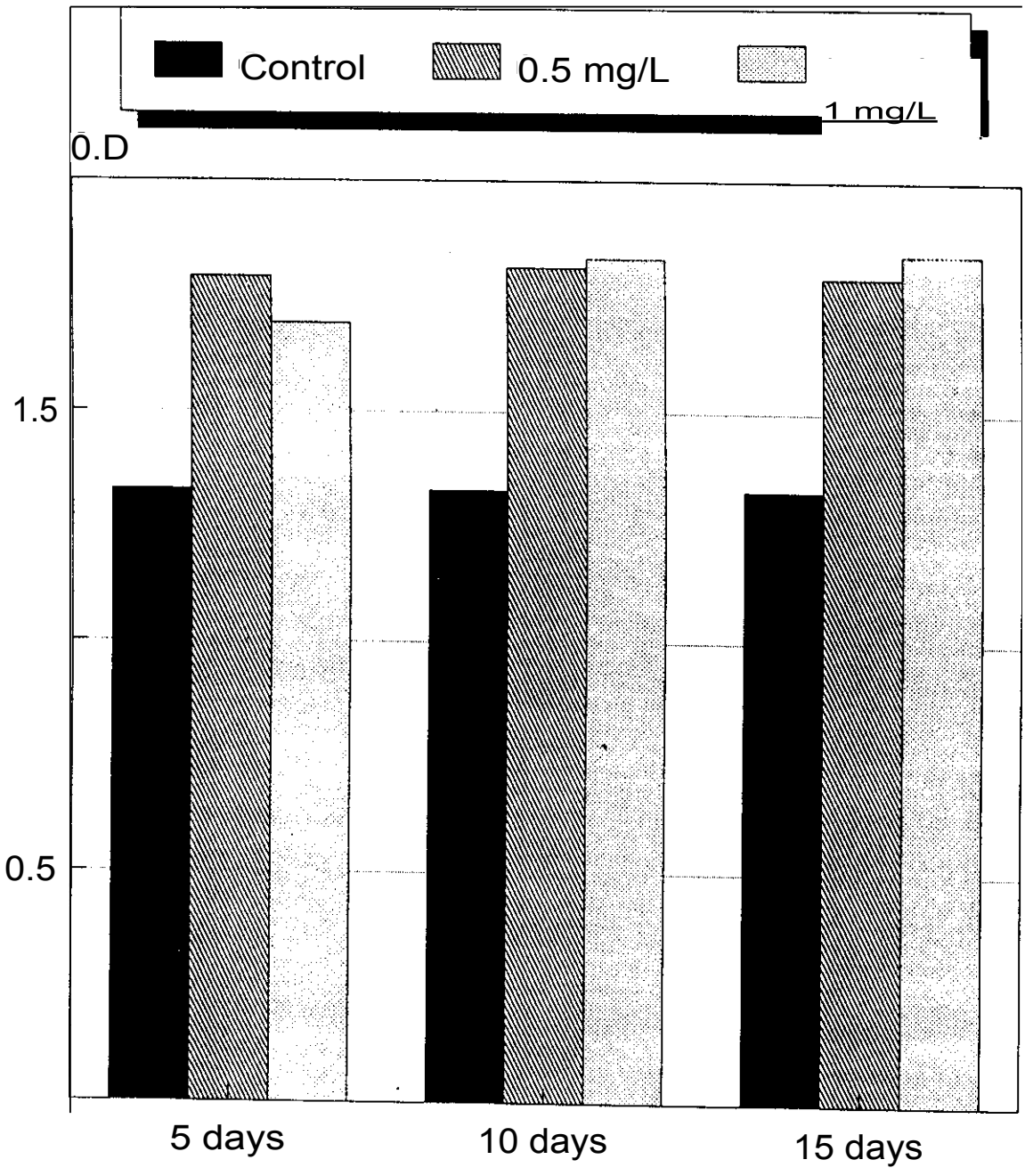


Fig.(142):The mean optical density value of aminot group content in muscle of **Oreochromis niloticus.**

Cytophotometric measurement of SS. group rich protein in muscle (table 20, Fig. 143) :

The 0.D values relative to the amount of SS. group containing proteins per cell as demonstrated by performic acid alcian blue stain is generally less in treated animal muscle fibres as compared to the control.

Treatment with 0.5 mg/L Sevin resulted in the following optical density values relative to SS content after 5 days (0.13 ± 0.02), 10 days (0.31 ± 0.03) and 15 days (0.26 ± 0.11).

Treatment with 1.0 mg/L resulted is value of (0.23 ± 0.01) at 5 days, (0.22 ± 0.06) at 10 days and (0.12 ±0.04) at 15 days as compared to the control for which the value was (0.27 ± 0.05).

Table (20): Effect of Sevin on SS. group rich protein content in muscle of 0. niloticus

Days		Control	0.5 mg/L	1.0 mg/L
5'	Mean ± S.D	0.27 0.05	0.13* 0.02	0.23* 0.01
10	Mean + S.D	0.27 0.05	0.31 0.03	0.22* 0.06
15	Mean ± S.D	0.27 0.05	0.26* 0.11	0.12 0.04

* Significant difference as compared to control at P < 0.025 and P < 0.05 (t) test.

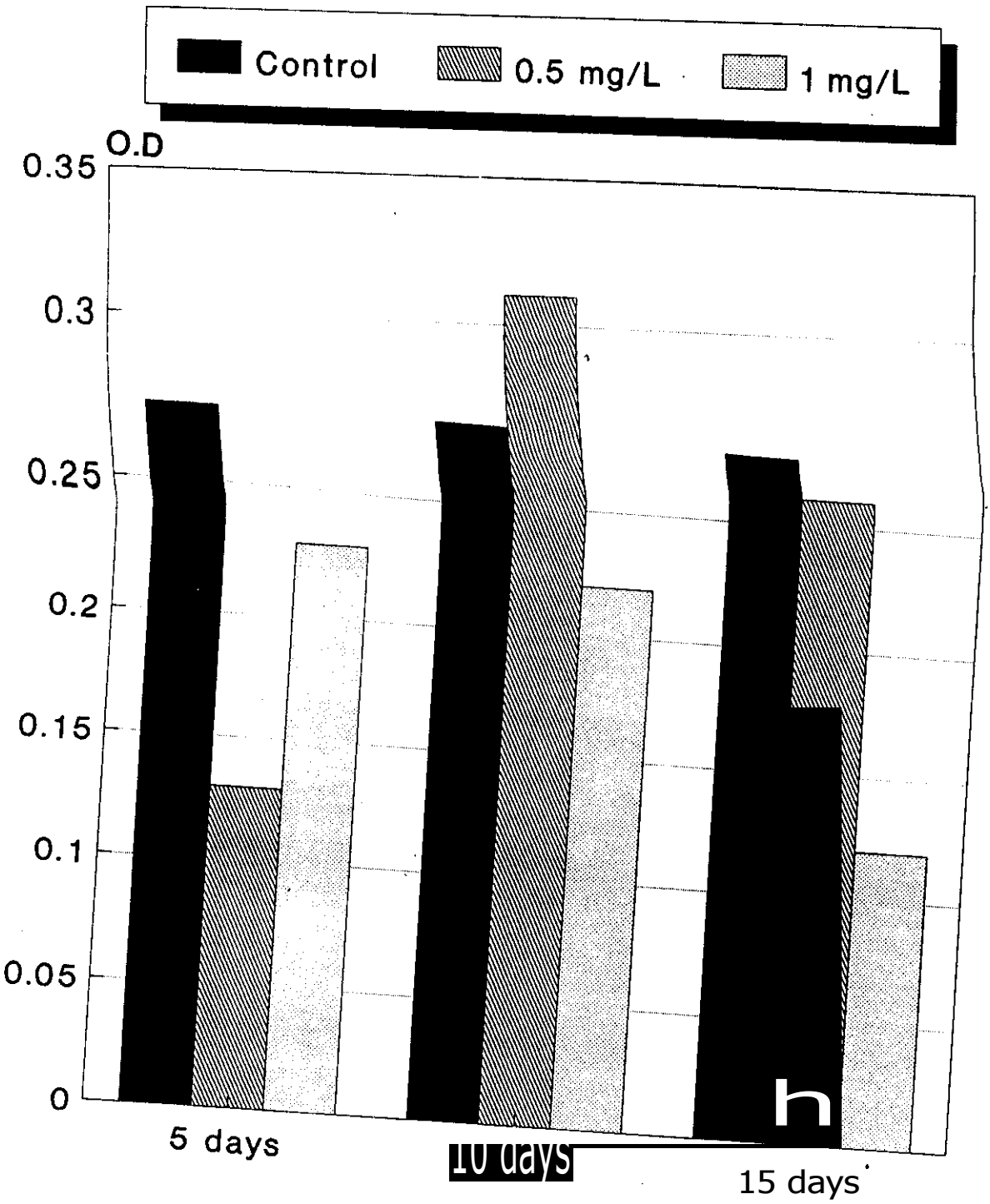


Fig.(143):The mean optical density value of SS group content in muscle of **Ore chromis niloticus●**